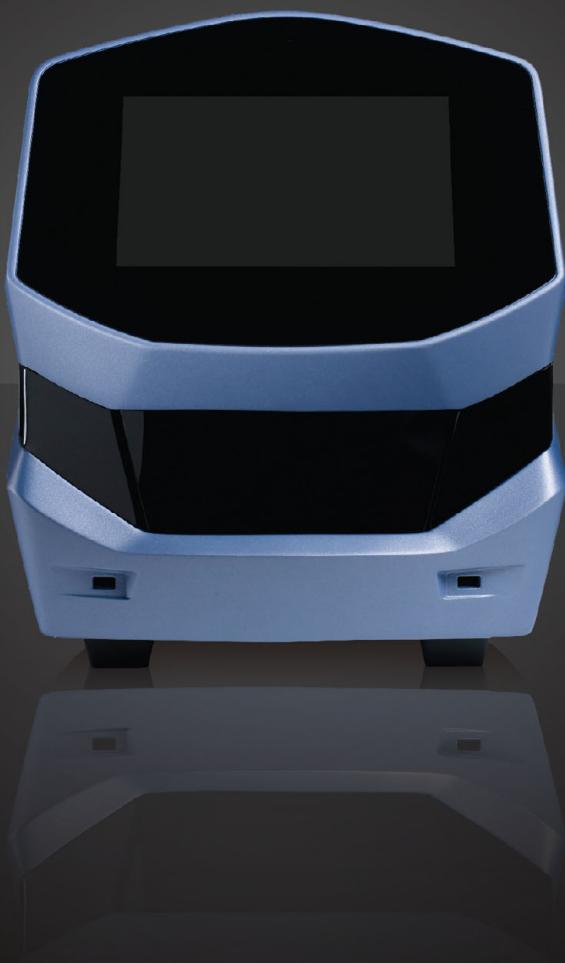


# Generate cDNA Libraries with the C1 Single-Cell mRNA Seq HT IFC and Reagent Kit v2

PROTOCOL



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# About This Guide

**IMPORTANT** Before using this kit, read and understand the detailed instructions and safety guidelines in this document. For complete safety information, see [Appendix G](#).

For detailed instructions on instrument and software operation, see the C1 System User Guide (PN 100-4977).

## Safety Alert Conventions

Fluidigm documentation uses specific conventions for presenting information that may require your attention. Refer to the following safety alert conventions.

### Safety Alerts for Chemicals

For hazards associated with chemicals, this document follows the United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS) and uses indicators that include a pictogram and a signal word that indicates the severity level:

Indicator	Description
	Pictogram (see example) consisting of a symbol on a white background within a red diamond-shaped frame. Refer to the individual safety data sheet (SDS) for the applicable pictograms and hazards pertaining to the chemicals being used.
<b>DANGER</b>	Signal word that indicates more severe hazards.
<b>WARNING</b>	Signal word that indicates less severe hazards.

### Safety Alerts for Instruments

For hazards associated with instruments, this document uses indicators that include a pictogram and signal words that indicate the severity level:

Indicator	Description
	Pictogram (see example) consisting of a symbol on a white background within a black triangle-shaped frame. Refer to the instrument user guide for the applicable pictograms and hazards pertaining to instrument usage.
<b>DANGER</b>	Signal word that indicates an imminent hazard that will result in severe injury or death if not avoided.
<b>WARNING</b>	Signal word that indicates a potentially hazardous situation that could result in serious injury or death if not avoided.

Indicator	Description
<b>CAUTION</b>	Signal word that indicates a potentially hazardous situation that could result in minor or moderate personal injury if not avoided.
<b>IMPORTANT</b>	Signal word that indicates information necessary for proper use of products or successful outcome of experiments.

## Safety Data Sheets

Read and understand the SDSs before handling chemicals. To obtain SDSs for chemicals ordered from Fluidigm, either alone or as part of this system, go to [fluidigm.com/sds](http://fluidigm.com/sds) and search for the SDS using either the product name or the part number.

Some chemicals referred to in this user guide may not have been provided with your system. Obtain the SDSs for chemicals provided by other manufacturers from those manufacturers.

# Introduction

## Overview of mRNA Seq HT Chemistry

This protocol details the use of the C1™ system and C1 high-throughput integrated fluidics circuits (HT IFCs) to capture up to 800 cells, apply a cell-specific barcode to all polyA+ RNA, convert polyA+ RNA into cDNA, and perform universal amplification of the cDNA for 3' end-counting mRNA sequencing (mRNA Seq)\* on Illumina® MiSeq, HiSeq, or NextSeq systems.

The protocol explains all steps performed, including capturing cells, staining cells for viability, imaging cells, lysing cells, barcoding, performing cDNA amplification by PCR, harvesting the amplified cDNA, multiplexing the cDNA, and generating 20 sequencing-ready cDNA libraries using a modified Illumina Nextera™ XT DNA sample preparation protocol.

**Figure 1** on page **8** shows how the 800 cells in the C1 mRNA Seq HT IFC are multiplexed. Cell barcodes are applied across each row of the HT IFC, and cDNA is harvested (pooled) through each of the 20 columns. Each column harvest contains barcoded cDNA from 40 cells. During library preparation, the Nextera index provides a second cell identifier, as shown in **Figure 2** on page **9**. Therefore, each cell is uniquely identified by a cell barcode applied on the HT IFC and a Nextera index during library preparation external to the HT IFC.

\* 3' end-counting is a technique used to determine the number of transcripts present per gene within an individual cell.

## Introduction

### Overview of mRNA Seq HT Chemistry

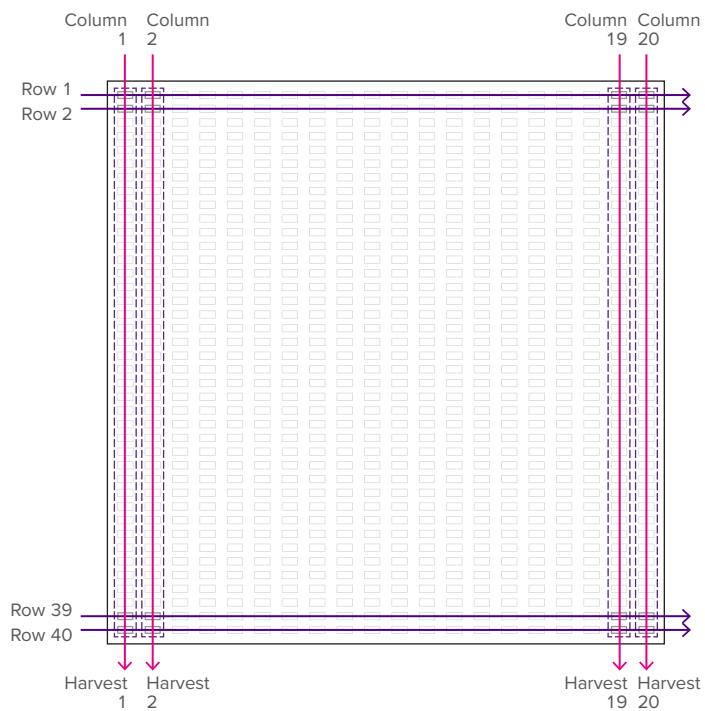


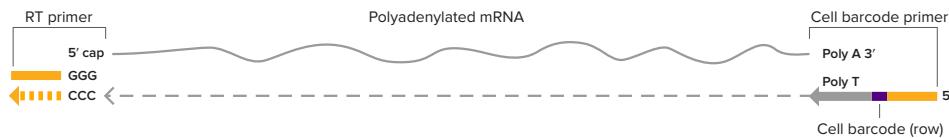
Figure 1. Overview of barcode arrangement for HT IFC harvest products (40 rows x 20 columns = 800 row-column barcode combinations)

The chemistry described in this protocol uses a modified oligo (dT) to prime first-strand cDNA synthesis by selecting for polyA+ RNA in a sample,\* while simultaneously integrating a cell barcode. When the reverse transcriptase reaches the 5' end of the mRNA, the enzyme's terminal transferase activity adds a few non-templated deoxycytidines to the 3' end of the cDNA. The reverse transcription (RT) primer contains a few guanosines at its 3' end that base-pair with the non-templated deoxycytidines on the cDNA to create an extended template. The reverse transcriptase extends to the end of the RT primer, producing single-stranded cDNA that contains a preamplification adapter, the 3' end of the cDNA, and the reverse complement of the preamplification adapter. Only polyadenylated RNA containing the preamplification adapter sequence at both ends will be amplified. Lastly, sequencing adapters and Nextera indices are applied during library preparation. In this reaction, only the 3' fragment of the transcript is enriched, therefore enabling 3' end-counting.

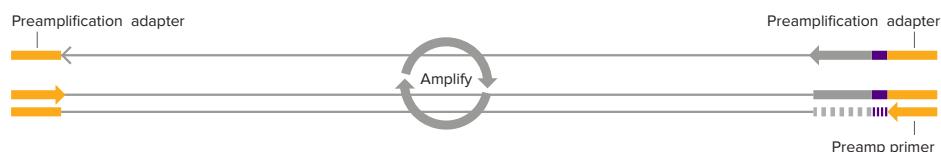
\* Chenchik, A., Zhu, Y.Y., Diatchenko, L. et al. "Generation and use of high-quality cDNA from small amounts of total RNA by SMART PCR," in *Gene Cloning and Analysis of RT-PCR*, BioTechniques Book (1998).

**Figure 2** shows an overview of the mRNA Seq HT chemistry used in this protocol. In this 3' end-counting approach, the cell barcode is applied across the row of the HT IFC during the reverse transcription step, while the Nextera index is used to define the column containing the cell during the 3' end enrichment (library preparation) step:

1. Reverse transcription



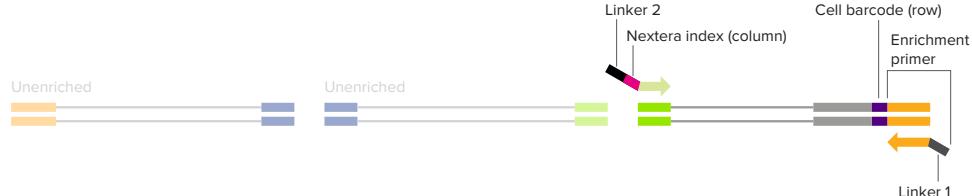
2. Preamplification



3. Library fragmentation



4. 3' end enrichment



5. Prepared next-generation sequencing template



6. Demultiplexed paired-end sequencing reads

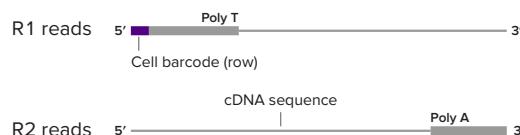
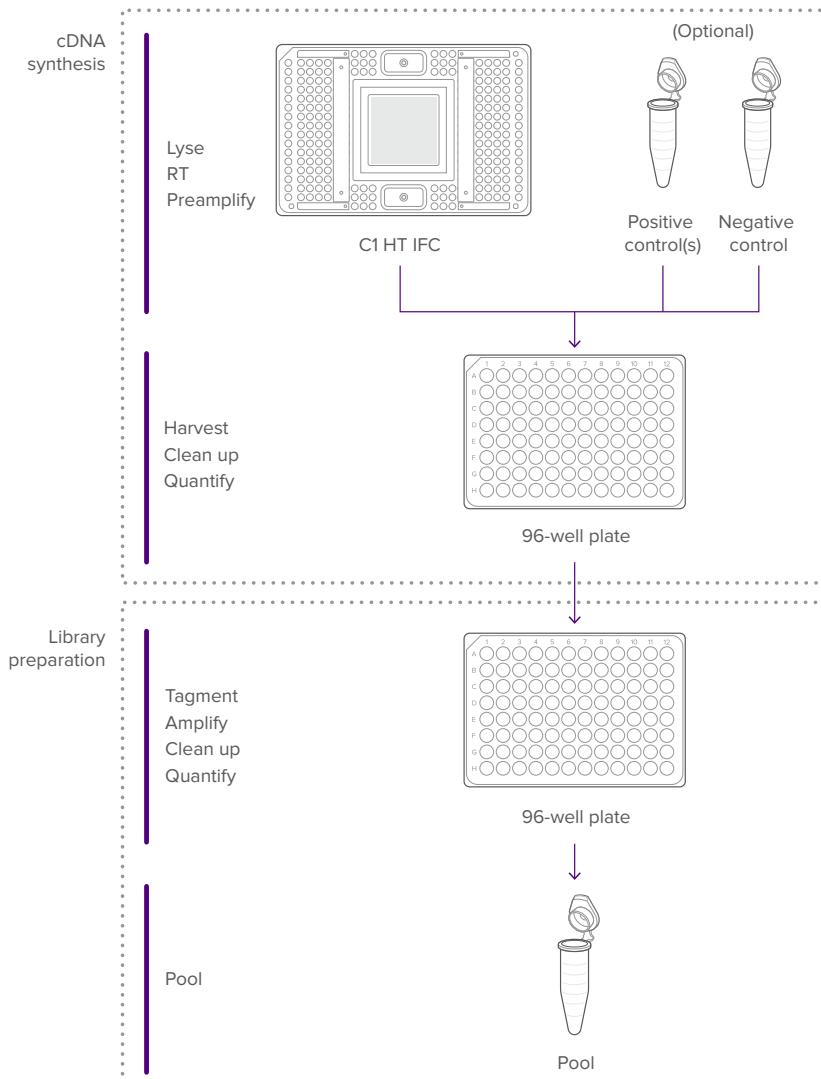


Figure 2. Overview of mRNA Seq HT chemistry

# Overview of HT Cell Capture Process

## Procedure Summary

During the HT cell capture process, you perform cDNA synthesis on the HT IFC and in optional tube controls, and library preparation off the HT IFC:



## Daily Workflow

An estimated timeline is shown below, but your actual timeline may vary. Incorporate additional time to the estimates below if you choose to run the optional tube controls (see [Appendix A](#) on page 67).

- **Day 1:** Cell capture, imaging, lysis, RT, and preamplification on the HT IFC
- **Day 2:** Harvest, cleanup, and library preparation off the HT IFC
- **Post-library preparation:** mRNA sequencing and data analysis

Reagent Handling	Automated Steps	Time
<b>Day 1: Cell capture, imaging, lysis, RT, and preamplification on the HT IFC</b>		
1 Dilute the 10X Blocking Reagent.		5 min
2 Pipet priming solutions into the HT IFC.		10 min
3	Prime the HT IFC on C1.	24 min  You have up to 60 minutes after the script finishes to load the HT IFC with the C1 system.
4 Prepare cells and optional viability stain during automated priming.		User-defined time, varies with cell type.
5 Pipet cells into the HT IFC.		1 min
6	Load cells on C1.	<ul style="list-style-type: none"><li>• 60 min if staining</li><li>• 40 min if not staining</li></ul>
7 Prepare reagent mixes A–C and diluted barcodes during automated cell loading.		60 min
8 Image cells with a microscope.		User-defined time, varies with microscope.
9 Pipet lysis, reverse transcription, and preamplification chemistry into the HT IFC.		10 min
10	Run the mRNA sequencing script on C1. This includes lysis, reverse transcription, PCR, and harvest.	8.5 hours  You can run the script overnight with a user-defined delay of harvest.  You have up to 60 minutes after the script finishes to transfer the cDNA from the HT IFC to a plate.

Reagent Handling	Automated Steps	Time
<b>Day 2: Harvest, cleanup, and library preparation off the HT IFC</b>		
<b>1</b> Transfer harvest amplicons from the HT IFC.		5 min
<b>2</b> Perform magnetic bead cleanup.		1.5–2 hours
<b>3</b> Quantify and dilute harvest amplicons.	Run Qubit® and optional Agilent® Bioanalyzer analysis.	60 min This time varies with optional Bioanalyzer analysis.
<b>4</b> Prepare and tagment the cDNA.		30 min
<b>5</b>	Amplify the tagmented cDNA on thermal cycler.	45 min
<b>6</b> Perform magnetic bead cleanup.		1.5–2 hours
<b>7</b> Quantify the library samples.	Run Qubit and optional Agilent Bioanalyzer® analysis.	60 min This time varies with optional Bioanalyzer analysis.
<b>8</b> Perform library pooling.		10 min
<b>Post-library preparation: mRNA sequencing and data analysis</b>		
<b>1</b> Prepare and load samples for sequencing.	Perform sequencing on Illumina MiSeq®, HiSeq, or NextSeq systems.	User-defined time, varies with system.
<b>2</b>	Run the C1 mRNA HT demultiplex script.	User-defined time, varies with number of samples.
<b>3</b>	Perform primary analysis (trim and align Illumina reads, and calculate gene expression).	User-defined time, varies with: <ul style="list-style-type: none"><li>• Number of samples</li><li>• Single-cell mRNA sequencing depth</li><li>• Server specifications and setup</li></ul>
<b>4</b>	Perform secondary analysis (differential gene expression analysis and visualizations).	User-defined time [see the Singular™ Analysis Toolset User Guide (version 3.5.x or later)].

# Materials

## Required Reagents

**IMPORTANT** Store reagents as soon as they are received, according to manufacturer's storage recommendations. The part numbers below provide the necessary reagents to run mRNA Seq HT chemistry on five C1 HT IFCs.

### Required Reagents from Fluidigm

The C1 Single-Cell mRNA Seq HT Reagent Kit v2 (C1 HT Kit) is shipped in five boxes (modules). When ordering the modules from Fluidigm, use the kit part number: 101-3473. For more information on the kit contents, see [Appendix D](#) on page 76.

Reagent	Part Number	Source	Storage
C1 Single-Cell mRNA Seq HT Reagent Kit v2	101-3473	Fluidigm	<ul style="list-style-type: none"><li>Module 1: 4 °C</li><li>Module 2: -20 °C</li><li>Module 3: -20 °C</li><li>Module 4: -80 °C</li><li>Module 5: -20 °C</li></ul>

## Materials

### Required Consumables

## Required Reagents from Other Suppliers

Reagent	Part Number	Source
SeqAmp™ DNA Polymerase	638504 (50 reactions)	Takara Bio, Inc.
Nextera XT DNA Library Preparation Kit (96 Samples)	FC-131-1096 <b>(Box 1 and Box 2)</b>	Illumina
Nextera XT Index Kit v2 (96 Indices, 384 Samples)	FC-131-2001 ( <b>Set A</b> ) and FC-131-2002 ( <b>Set B</b> )	
Qubit dsDNA HS Assay Kit	Q32851 (100 assays) or Q32854 (500 assays)	Thermo Fisher Scientific
Agencourt® AMPure XP	A63880 (5 mL) or A63881 (60 mL)	Beckman Coulter
<b>NOTE</b> You will need three bottles of PN A63880 or one bottle of PN A63881 to run five C1 HT IFCs with this protocol.		
DNA Suspension Buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0)*	T0227 (100 mL)	Teknova
Ethanol, 200 proof	—	Major laboratory supplier (MLS)

\* Recommended if Teknova is not available in your location: 1X TE Buffer (Thermo Fisher Scientific, PN 12090015).

## Required Consumables

Product	Part Number	Source
C1 Single-Cell mRNA Seq HT IFC (10–17 µm)	101-4981 (1 IFC) or 101-4982 (5 IFCs)	Fluidigm
High Sensitivity DNA Kit	5067-4626	Agilent Technologies
Qubit Assay Tubes	Q32856 (500 tubes)	Thermo Fisher Scientific
MAXYMum Recovery™ Microtubes (1.5 mL)	MCT-150-L-C	Axygen Scientific
MicroAmp® Clear Adhesive Film	4306311	Thermo Fisher Scientific
PCR Sealing Film	82018-844	VWR International
96-well PCR plates*	—	MLS
0.2 mL PCR 8-tube strips	—	MLS
Filtered pipette tips	—	MLS
Low-lint cloth	—	MLS

\* Recommended: TempPlate® semi-skirted 96-well PCR plates (USA Scientific PN 1402-9700).

## Required Equipment

Product	Part Number	Source
C1 system	100-7000	Fluidigm
2100 Bioanalyzer*	G2940CA	Agilent Technologies
Qubit 3.0 Fluorometer	Q33216	Thermo Fisher Scientific
96-well PCR plate thermal cycler	–	MLS
Three centrifuges: one picofuge, one for microtubes, one for 96-well PCR plates	–	MLS
Vortexer	–	MLS
Select the appropriate magnet for your 96-well PCR plate:		Thermo Fisher Scientific
<ul style="list-style-type: none"> <li>• DynaMag™-96 Side Magnet (<b>recommended</b>) – for use with semi-skirted plates (see page 14) and with non-skirted plates</li> <li>• DynaMag™-96 Side Skirted Magnet – for use with full-skirted plates</li> </ul>	<ul style="list-style-type: none"> <li>• 12331D or</li> <li>• 12027</li> </ul>	
<b>IMPORTANT</b> PCR plates may vary. Make sure to test your plate for compatibility before use.		
Magnetic stand for microtubes <sup>†</sup>	–	MLS

\* Recommended: At least two Bioanalyzers to minimize the time required to complete this protocol (see [page 12](#)).

<sup>†</sup> Recommended: DynaMag™-2 Magnet (Thermo Fisher Scientific PN 12321D).

## Suggested Reagents

Reagent	Part Number	Source
ArrayControl™ RNA Spikes	AM1780	Thermo Fisher Scientific
The RNA Storage Solution (10 × 1 mL)	AM7000	
LIVE/DEAD® Viability/Cytotoxicity Kit, for mammalian cells	L-3224	
PhiX Control v3	FC-110-3001	Illumina
1X Phosphate-buffered saline (PBS) or similar wash buffer	–	MLS

## Suggested Consumables

Product	Part Number	Source
C-Chip™ Disposable Hemocytometer (Neubauer Improved)	DHC-N01	INCYTO
Corning® Black Polystyrene Universal Microplate Lid with Corner Notch, Sterile (50 lids/case)	3935	Corning

## Suggested Equipment

Product	Part Number	Source
Two hoods (DNA and DNA-free)*	–	MLS
Imaging equipment compatible with C1 mRNA Seq HT IFCs <sup>†</sup>	–	MLS

\* To prevent DNA contamination of lab and samples.

<sup>†</sup> See the Minimum Specifications for Single-Cell Imaging Specification Sheet (PN 100-5004).

# Before You Begin

To ensure reliable results, we recommend that you do the following:

- Make sure to install C1 system software v2.2.3 or later to run the latest scripts. For more information, see the C1 System Software Release Notes (PN 101-5841) and Updating the C1 System Software Quick Reference (PN 100-6217).
- Use good laboratory practices to minimize contamination of samples. Use a new pipette tip for every new sample. Change gloves **before and after** handling the cell barcodes (see page [31](#) for barcode preparation).
- Establish a working cell dissociation protocol for each new cell type you will run on the HT IFC before proceeding with this protocol (see page [26](#) for cell mix requirements).
- Practice microscopy imaging before loading the HT IFC for the first time (see page [34](#) for cell imaging recommendations). If you practice with an unused IFC, cover the IFC with PCR sealing film to prevent contamination during imaging.
- Use the pipetting maps provided with this protocol (see page [22](#) for the first map). Follow the instructions for each map, and make sure to remove any remaining reagents before loading the reagents in the order shown. (See the Pipetting Maps for the C1 mRNA Seq HT v2 Protocol: Quick Reference (PN 101-4965) for all maps in an actual size, printable format. If necessary, transparent tape (such as Scotch™ tape) or a Corning® black plate lid (Corning PN 3935) can also be used to anchor the IFC to the printed map and to assist IFC pipetting.)

# Generate High-Throughput Single-Cell cDNA Libraries for mRNA Sequencing

## Retrieve the Reagents for cDNA Synthesis

**IMPORTANT** To ensure reliable results:

- Retrieve only the reagents required from each kit based on the number of HT IFCs you will run in your daily workflow (see page 11). Only use the reagents provided in the required kit. Do not swap reagents between kits.
- Thaw reagents on ice unless directed to thaw them at room temperature. Mix and centrifuge reagents as directed. **Do not vortex** reagents unless directed. **Avoid creating bubbles.**

Workflow Step	Source	Required Reagent	Quantity (for 1 IFC)	Preparation
1 HT IFC priming (see page 20)	C1 HT Kit, Module 1	Valve Fluid v2		1 tube
		Actuation Fluid		1 tube
		10X Blocking Reagent		1 tube
		Stability Solution		1 tube
		Cell Rinsing Reagent		2 tubes
2 (Optional) RNA Spikes Mix (see page 29)	ArrayControl RNA Spikes	ArrayControl RNA Spikes	1 tube	Remove from -80 °C, thaw on ice, and keep on ice
	The RNA Storage Solution	The RNA Storage Solution	1 bottle	Keep at room temperature
3 (Optional) RNA Spikes Mix dilution (see page 30)	C1 HT Kit, Module 5	RNase Inhibitor		1 tube
	C1 HT Kit, Module 2	Preloading Reagent		2 tubes
4 Lysis Mix—Mix A (see page 31)	C1 HT Kit, Module 5	10X Lysis Buffer - v3		Remove from -20 °C, thaw on ice, and keep on ice ( <b>do not vortex</b> )
		Nuclease-Free Water		Remove from -20 °C and thaw to room temperature

Workflow Step	Source	Required Reagent	Quantity (for 1 IFC)	Preparation
5 Lysis Mix A plus diluted barcodes (see page 31)	C1 HT Kit, Module 3	Cell Barcode Plate	1 plate	Remove from -20 °C, thaw on ice, and keep on ice ( <b>do not vortex</b> )
	Teknova	DNA Suspension Reagent	1 bottle	Keep at room temperature
6 Reverse Transcription Mix—Mix B (see page 33)	C1 HT Kit, Module 4	Reverse Transcription Primer	1 tube	Remove from -80 °C, thaw on ice, and keep on ice
	C1 HT Kit, Module 2	Loading Reagent	1 tube	Remove from -20 °C and thaw to room temperature
	C1 HT Kit, Module 5	5X First-Strand Buffer	1 tube	Remove from -20 °C, thaw on ice, and keep on ice
		Dithiothreitol (DTT)	1 tube	
		dNTP Mix (20 mM each)	1 tube	
		Reverse Transcriptase	1 tube	Remove from -20 °C, thaw on ice, and keep on ice ( <b>do not vortex</b> )
7 Preamplification Mix—Mix C (see page 34)	SeqAmp DNA Polymerase	SeqAmp PCR Buffer (2X)	1 tube	Remove from -20 °C, thaw on ice, and keep on ice
		SeqAmp DNA Polymerase	1 tube	Remove from -20 °C, thaw on ice, and keep on ice ( <b>do not vortex</b> )
	C1 HT Kit, Module 2	Preamp Primer	1 tube	Remove from -20 °C, thaw on ice, and keep on ice
8 (Optional) LIVE/DEAD cell staining (see page 25)	LIVE/DEAD Kit	Ethidium homodimer-1	1 tube	Remove from -20 °C and thaw to room temperature (keep in the dark as much as possible)
		Calcein AM	1 tube	
	C1 HT Kit, Module 1	Cell Rinsing Reagent	1 tube	Remove from 4 °C and equilibrate to room temperature
9 Cell loading (see page 24)	User-supplied	User-supplied cells	1 or 2 cell types	Prepare, count, and resuspend to appropriate concentration (see page 26; <b>do not vortex</b> )
	C1 HT Kit, Module 1	Suspension Reagent	1 tube	Remove from 4 °C and vortex well
10 HT IFC run chemistry (see page 36)	C1 HT Kit, Module 2	C1 Harvest Reagent	1 bottle	Remove from -20 °C and thaw to room temperature
11 Cell wash for optional tube controls (see page 67)	User-supplied	1X PBS (or similar wash buffer)	1 bottle	Keep at room temperature

## Prime the HT IFC

### Dilute the Blocking Reagent

Prepare two dilutions of the 10X Blocking Reagent before priming the HT IFC:  
1X and 0.01X.

#### 1X Blocking Reagent

- 1 Combine the following reagents in a microtube:

Table 1. 1X Blocking Reagent dilution

Reagent		Volume ( $\mu$ L)
Cell Rinsing Reagent (C1 HT Kit Module 1)		135
10X Blocking Reagent (C1 HT Kit Module 1)		15
<b>Total</b>		<b>150</b>

- 2 Gently vortex and centrifuge briefly to collect contents. Keep at room temperature.

#### 0.01X Blocking Reagent

- 1 Combine the following reagents in a microtube:

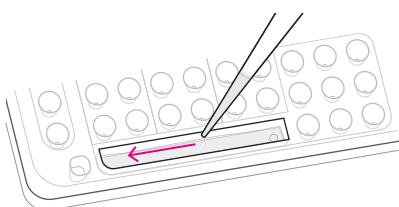
Table 2. 0.01X Blocking Reagent dilution

Reagent		Volume ( $\mu$ L)
Cell Rinsing Reagent (C1 HT Kit Module 1)		396
1X Blocking Reagent dilution (from step 2 above)		4
<b>Total</b>		<b>400</b>

- 2 Gently vortex and centrifuge briefly to collect contents. Keep at room temperature.

## Before You Pipet into the HT IFC

- Ensure that the notch (A1 position) is at the top-left corner of the HT IFC and the barcode faces to the left.
- Always stop at the first stop on the pipette to avoid creating bubbles in the HT IFC inlets. If a bubble is introduced, ensure that it floats to the top of the well.
- The viscosity of some solutions may vary. Pipet high viscosity solutions slowly and carefully to avoid bubbles. Pipet low viscosity solutions quickly and carefully to avoid leaks into unintended HT IFC inlets.
- Make sure to keep the HT IFC as level as possible, and evenly distribute reagents over the bottom surfaces of the accumulators and reservoirs.
- To evenly distribute reagents and avoid creating bubbles in reservoirs, pipet outward from edge closest to the center of the HT IFC and do not allow a pipette tip to touch the inlet hole (→).



## Prime the HT IFC

Load the reagents as shown in [Figure 3](#).

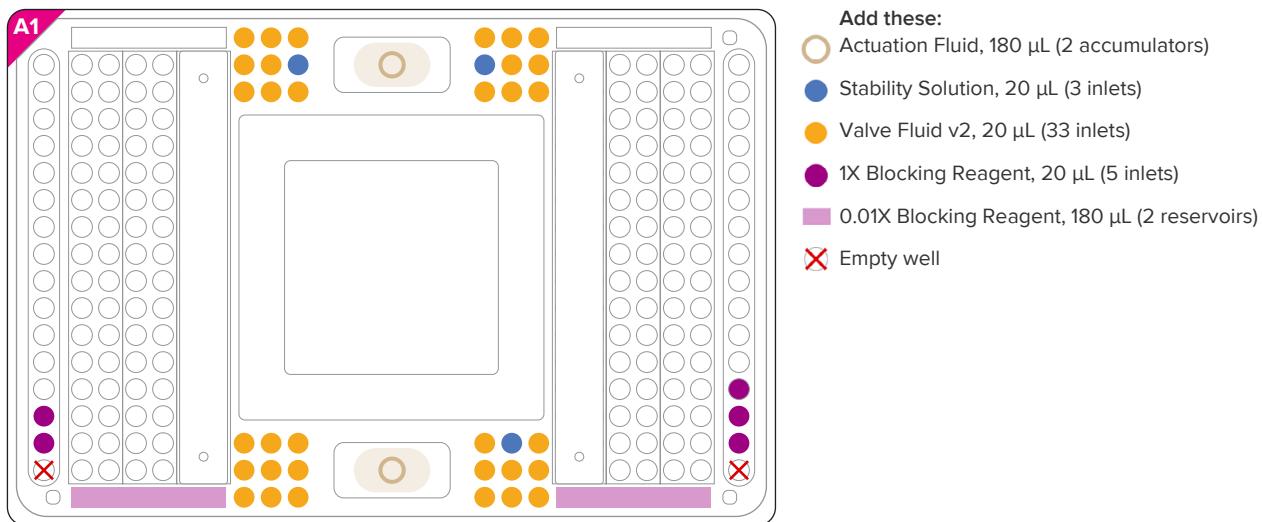


Figure 3. HT IFC priming pipetting map

- 1 Peel off white tape on bottom of HT IFC.

**IMPORTANT** For best results, we recommend that you place the appropriate pipetting map under the HT IFC to use as a pipetting guide. See the Pipetting Maps for the C1 mRNA Seq HT v2 Protocol: Quick Reference (PN 101-4965) for all maps in an actual size, printable format. If necessary, transparent tape (such as Scotch™ tape) or a Corning® black plate lid (Corning PN 3935) can also be used to anchor the IFC to the printed map and to assist IFC pipetting.

- 2 Using a P200 pipette, carefully pipet 180 µL of Actuation Fluid into each of the accumulators (tan outlined circles ○), **avoiding spills**. Use the pipette tip to gently press down on the black O-ring, insert the tip to one side of the accumulator, and then release the fluid.

**IMPORTANT** Actuation Fluid is a low surface tension solution. Make sure to use a P200 pipette. Make sure to keep the HT IFC as level as possible, and evenly distribute the Actuation Fluid over the bottom surface of the accumulators. Use a lint-free wipe to remove any excess fluid around the accumulator.

- 3 Using a P200 pipette, **slowly** and carefully pipet 20 µL of Stability Solution into each of the 3 inlets (blue circles ●), **avoiding spills**.

**IMPORTANT** The Stability Solution is viscous. **Do not vortex**. Make sure to use a P200 pipette.

- 4 Using a P20 pipette, pipet 20 µL of Valve Fluid v2 into each of the 33 control line inlets near the accumulators (solid gold circles ○).

- 5** Make sure to dilute the Blocking Reagent (see page [20](#)) and then:
  - a Pipet 20 µL of 1X Blocking Reagent into each of the 5 inlets (solid dark violet circles ●).
  - b Pipet 180 µL of 0.01X Blocking Reagent into each of the 2 wash reservoirs at the bottom of the HT IFC (solid light violet rectangles ■).
- IMPORTANT** Make sure to keep the HT IFC as level as possible, and evenly distribute the 0.01X Blocking Reagent over the bottom surface of the reservoirs.
- 6** Place the IFC into the C1 system, and then run the **mRNA Seq HT: Prime (1912x)** script. Priming takes approximately 24 minutes.

**NOTE**

- Make sure to install C1 system software v2.2.3 or later to run the latest scripts. For more information, see the C1 System Software Release Notes (PN 101-5841) and Updating the C1 System Software Quick Reference (PN 100-6217).
- See the C1 System User Guide (PN 100-4977) for instructions for use. If a priming reagent pipetting error has occurred, wait for the Prime script to finish (**do not** press ABORT), pipet new priming reagents, and then run the prime script again.

- 7** During HT IFC priming, prepare the cell mix for loading (see [Prepare and Load Cells](#) on page [24](#)). If time allows, you can also start preparing the reagent mixes and diluted barcodes (see [Prepare Reagent Mixes for cDNA Synthesis](#) on page [29](#)).

**NOTE** After priming the HT IFC, you have up to 1 hour to load cells. We recommend that you keep the primed IFC in the instrument until you are ready to load cells.

- 8** When the Prime script has finished, tap **EJECT** to remove the primed IFC from the instrument.

## Prepare and Load Cells

The center panel of the HT IFC is divided into two sections, left and right, with 10 columns of 40 capture sites in each section. You can load the same or different cell mixes into each section through the corresponding cell inlets:

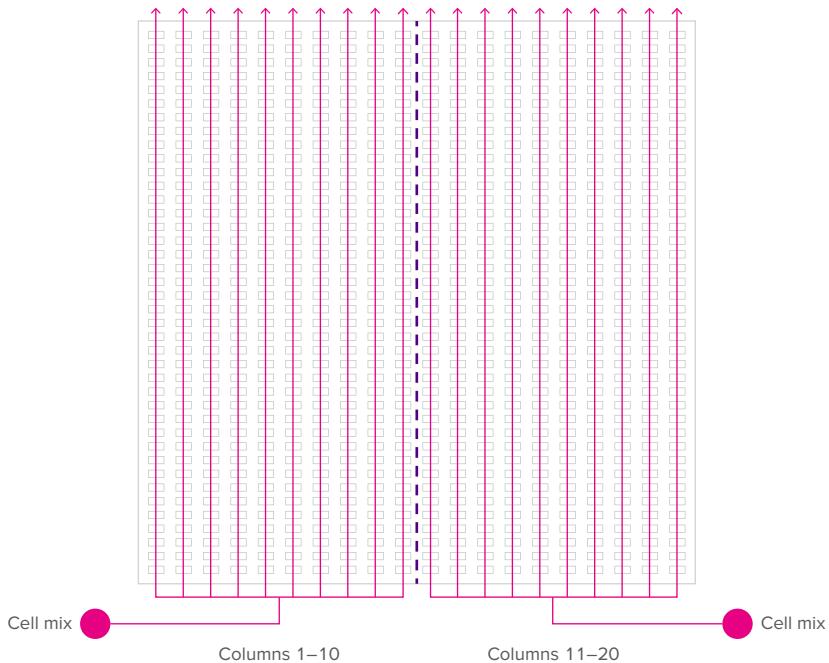


Figure 4. Cell loading pattern in the center panel of the HT IFC

Follow these protocols to prepare the cell mix for loading into the HT IFC:

- (Optional) Prepare LIVE/DEAD Cell Staining Solution on page [25](#)
- Prepare the Cell Mix While Priming the HT IFC on page [26](#)

## (Optional) Prepare LIVE/DEAD Cell Staining Solution

### About the Dyes

The LIVE/DEAD Viability/Cytotoxicity Kit tests the viability of a cell based on the integrity of the cell membrane. This test contains two chemical dyes. The first dye is green-fluorescent calcein AM, which stains live cells. This dye is cell-permeable and tests for active esterase activity in live cells. The second dye is red-fluorescent ethidium homodimer-1, which will stain cells only if the integrity of the cell membrane has been lost.

### Prepare the Cell Staining Solution

Cell staining solution may be prepared up to 2 hours before loading into the HT IFC.

**IMPORTANT** Keep the dye tubes closed and in the dark as much as possible, because they can hydrolyze over time. When not in use, store in dark, airtight bag with desiccant pack at -20 °C.

- 1 Vortex the dyes for 10 seconds and then centrifuge them before pipetting.
- 2 Prepare the LIVE/DEAD staining solution by combining reagents in this order:

Components	Volume (µL)
Cell Rinsing Reagent (C1 HT Kit Module 1)	1,250.0
Ethidium homodimer-1 (Thermo Fisher Scientific)	2.5
Calcein AM Thermo Fisher Scientific	0.625
<b>Total</b>	<b>1,253.125</b>

- 3 Keep the staining solution on ice and protected from light until use. Vortex the solution well before pipetting into the HT IFC.

## Prepare the Cell Mix While Priming the HT IFC

You can load the same or two different cell mixes into the HT IFC.

### Prepare the Single-Cell Suspension

**IMPORTANT** Establish a working cell dissociation protocol for each new cell type you will run on the HT IFC before proceeding with this protocol. For more information, see the Fluidigm Single-Cell Preparation Guide (PN 100-7697).

- 1 Prepare a cell suspension of 400 cells/ $\mu$ L for medium cells (10–17  $\mu$ M) in native medium using an established protocol for your cell type.

**NOTE** Cells may be counted by any preferred method. If an established cell counting protocol does not exist, we suggest using the disposable hemocytometer C-Chip by INCYTO. See [incyto.com/product/product02\\_detail.php](http://incyto.com/product/product02_detail.php) for instructions for use.

- 2 Keep the cell suspension at the appropriate storage condition for your cell type.

### Prepare the Cell Mix for the HT IFC

Prepare the cell mix by combining cells with Suspension Reagent at a ratio optimized in advance for your cell type, to create a neutrally buoyant cell suspension for loading into the HT IFC.

**IMPORTANT** Vortex the Suspension Reagent ● for 5 seconds before use. If Suspension Reagent contains particulates, ensure they are properly removed by vortexing. **Do not vortex** the cells.

- 1 Use a P200 pipette to **gently and slowly** add the cells to the Suspension Reagent in a microtube. Many cell types use the standard suspension ratio of 3:2 as shown below.

Components	Volume for each cell inlet ( $\mu$ L)
Suspension Reagent (C1 HT Kit Module 1)	20
Cells (from step 1 above)	30
<b>Total</b>	<b>50</b>

**NOTE** The volume of cell mix may be scaled depending on volume of cells available. You will load 10  $\mu$ L of cell mix into each cell inlet in the HT IFC (see [Figure 6](#)).

- 2 (Optional) If performing a tube control, label the tube containing the remaining cells “PC” and keep at the appropriate storage condition for your cell type, for later use in the tube control reactions (see [Wash the Cells](#) on page [67](#)).

## Load Cells

### Step 1. Remove Reagents

Before you continue, make sure to remove the reagents as shown in [Figure 5](#).

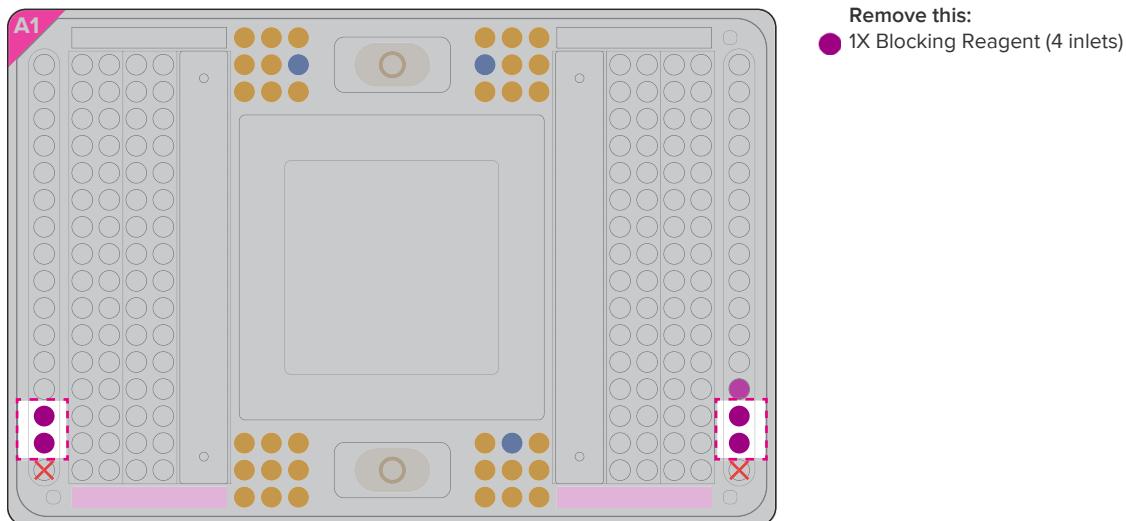


Figure 5. HT IFC remove before loading pipetting map

Use a P20 set to 20  $\mu\text{L}$  to remove the 1X Blocking Reagent from each of the 4 inlets (solid dark violet circles ●).

**NOTE** After priming the IFC, you might see residual 1X Blocking Reagent in the remaining ● inlet. You do not need to remove it.

## Step 2. Load Reagents

Before you continue, make sure to remove the reagents as shown in [Figure 5](#), and then proceed to load the reagents as shown in [Figure 6](#).

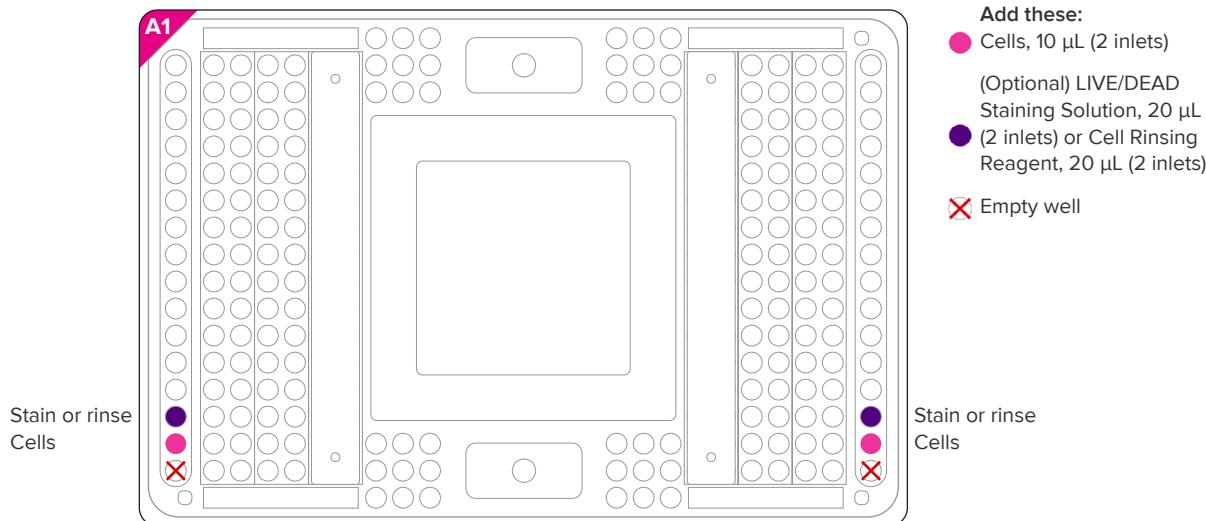


Figure 6. HT IFC loading pipetting map

- 1 Set a P200 pipette to 90% of the total cell mix volume (see step 1 on page [26](#)), and then slowly pipet the cell mix up and down 3–5 times to mix, depending on whether the cells tend to clump. **Do not vortex. Avoid creating bubbles.**
  - 2 Pipet 10 µL of cell mix into the 2 cell inlets (solid magenta circles ●).
  - NOTE** You can load the same cell mix into both cell inlets, or a different cell mix into each cell inlet (see [Figure 4](#) on page [24](#)).
  - 3 Pipet one of the following into the 2 inlets labeled “stain or rinse” (solid purple circles ●):
    - Staining cells:** Vortex the LIVE/DEAD staining solution well, and then pipet 20 µL of the staining solution into the 2 stain inlets (●).
    - Not staining cells:** Pipet 20 µL of Cell Rinsing Reagent into the 2 stain inlets (●).
  - NOTE** If staining only one cell mix, make sure to pipet the LIVE/DEAD stain and Cell Rinsing Reagent into the respective sections of the HT IFC (left or right), and run the **Cell Load & Stain** script (see step 4).
  - 4 Place the HT IFC into the C1 system. Run the **mRNA Seq HT: Cell Load (1912x)** or **mRNA Seq HT: Cell Load & Stain (1912x)** script. Approximate times: cell loading takes 40 minutes and cell loading with staining takes 60 minutes.
  - 5 During cell loading, you can prepare reagent mixes (see below).
  - 6 When the script has finished, tap **EJECT** to remove the HT IFC from the C1 system.
- NOTE** If you stained the cells, keep the HT IFC in the dark as much as possible before imaging.

# Prepare Reagent Mixes for cDNA Synthesis

During cell loading and imaging, prepare the following reagent mixes for lysis, RT, and preamplification:

- (Optional) ArrayControl RNA Spikes
- Lysis Mix—Mix A on page 31
- Lysis Mix A Plus Diluted Barcodes on page 31
- Reverse Transcription (RT) Reaction Mix—Mix B on page 33
- Preamplification Mix—Mix C on page 34

## (Optional) ArrayControl RNA Spikes

RNA spikes serve as a positive control for thermal cycling of the C1 system that is independent of cell capture. Although this control is not required, it is highly recommended.

### NOTE

- Due to the low volume pipetted, we highly recommend making the ArrayControl RNA Spikes mix in bulk and aliquoting for future use.
- ArrayControl RNA Spikes contain eight RNA transcripts. We will use only three.

### Prepare the RNA Spikes Mix

- 1 After the ArrayControl RNA Spikes have thawed, remove spikes 1, 4, and 7 from the box.
- 2 Combine the following reagents in three separate microtubes:

Tube	A	B	C
The RNA Storage Solution	13.5 µL	12.0 µL	148.5 µL
RNA Spikes	No. 7: 1.5 µL	No.4: 1.5 µL	No. 1: 1.5 µL

- 3 Vortex tube A for 3 seconds and centrifuge to collect contents. Pipet 1.5 µL from tube A into tube B. Discard tube A.
- 4 Vortex tube B for 3 seconds and centrifuge to collect contents. Pipet 1.5 µL from tube B into tube C. Discard tube B.
- 5 Vortex tube C for 3 seconds and centrifuge to collect contents. Tube C is the concentrated RNA standard that may be aliquoted and frozen for future use.

- 6** Aliquot 1.25 µL into separate microtubes and store at –80 °C until use. One tube is necessary for each C1 run.

### Dilute the RNA Spikes Mix for the Lysis Mix (Mix A)

**NOTE** Diluted RNA does not store well. Do not dilute RNA more than an hour before you load the HT IFC. Only store concentrated aliquots long term.

- 1** Thaw an RNA Spikes Mix aliquot (from step 6 above).

- 2** Dilute by combining:

Table 3. RNA Spikes Mix dilution

Reagent	Volume (µL)
Preloading Reagent (C1 HT Kit Module 2)	28.7
RNAse Inhibitor (C1 HT Kit Module 5)	1.0
RNA Spikes Mix aliquot (from step 6 above)	1.0
<b>Total</b>	<b>30.7</b>

- 3** Vortex for 3 seconds and centrifuge briefly to collect contents.

## Lysis Mix—Mix A

You can prepare Lysis Mix with or without the optional RNA spikes.

- 1 Combine the following reagents in a microtube labeled “A.”

Components		Volume (without RNA spikes; $\mu\text{L}$ )	Volume (with RNA spikes; $\mu\text{L}$ )
Nuclease-Free Water (C1 HT Kit Module 5)	●	206.4	193.7
10X Lysis Buffer - v3 (do not vortex) (C1 HT Kit Module 5)	●	45.6	45.6
RNase Inhibitor (C1 HT Kit Module 5)	○	2.4	2.4
(Optional) RNA Spikes Mix dilution (from <a href="#">Table 3</a> on page <a href="#">30</a> )		—	12.72
<b>Total</b>		<b>254.4</b>	<b>254.4</b>

- 2 Gently pipet up and down a few times to mix (**do not vortex**). **Avoid creating bubbles**. Centrifuge briefly to collect contents. Keep on ice until use.

## Lysis Mix A Plus Diluted Barcodes

The Cell Barcode Plate contains concentrated cell (row) barcodes that are **single-use only** (see page [77](#) for the plate map). You must first prepare a diluted barcodes plate, then prepare a plate containing Lysis Mix A plus the diluted barcodes as shown in [Figure 7](#).

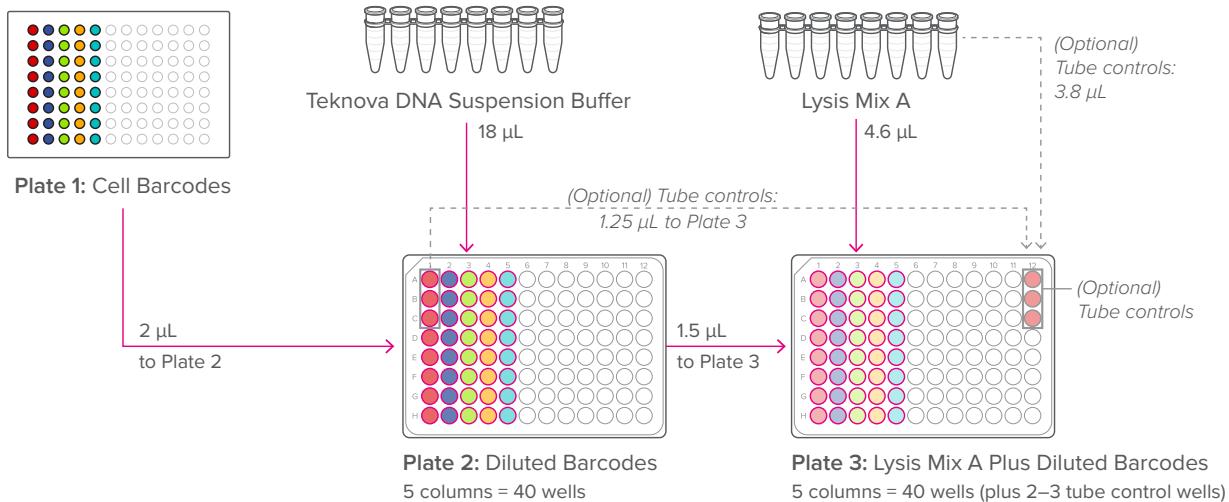
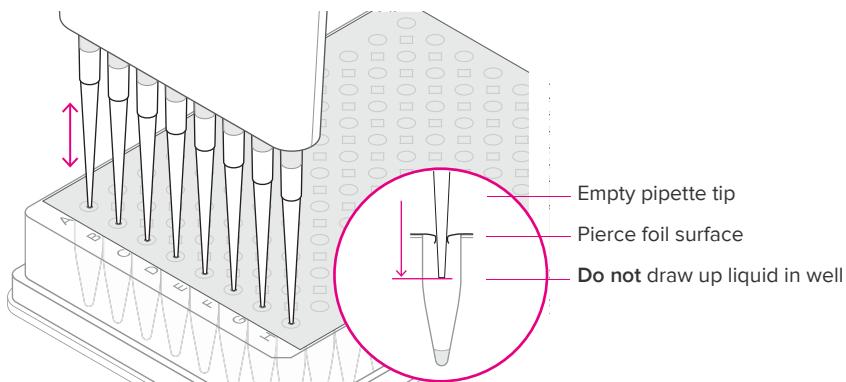


Figure 7. Cell barcode dilution process

**IMPORTANT** To avoid contamination: do not remove the foil cover on the Cell Barcode Plate, do not vortex plates containing barcodes, use fresh pipette tips for each well, and change gloves before and after handling barcodes.

### Prepare Plate 2: Diluted Barcodes

- 1 Centrifuge the original Cell Barcode Plate at  $\sim 3,000 \times g$  for 3 minutes (do not vortex to avoid contamination). This is Plate 1.
- 2 Label a new 96-well PCR plate “Diluted Barcodes”. This is Plate 2.
- 3 Aliquot 100  $\mu\text{L}$  of Teknova DNA Suspension Buffer into each tube of an 8-tube strip, then use a multichannel pipette to pipet 18  $\mu\text{L}$  of the buffer into the first 5 columns of the Diluted Barcodes plate (40 wells total).
- 4 Using a multichannel pipette with fresh P200 pipette tips for each well, pierce the foil for the first 5 columns of the Cell Barcode Plate. **Do not remove the foil and do not pipet any liquid with the P200 pipette** to avoid contamination.



- 5 Using a P20 or P10 pipette, pipet 2  $\mu\text{L}$  of the barcodes column by column into the same 40 wells of the Diluted Barcodes plate and pipet up and down 10 times to mix. **Do not vortex** and **pipet carefully** to avoid contamination. Keep the Diluted Barcodes plate on ice until use in step 3 on page 33.

### Prepare Plate 3: Lysis Mix A Plus Diluted Barcodes

- 1 Label a new 96-well PCR plate “Lysis Mix A Plus Diluted Barcodes”. This is Plate 3.
- 2 Aliquot 30  $\mu\text{L}$  of Lysis Mix A into each tube of an 8-tube strip, and then carefully pipet the following, **avoiding bubbles**:
  - For samples – Pipet 4.6  $\mu\text{L}$  of Lysis Mix A into the first 5 columns of the **new** Lysis Mix A Plus Diluted Barcodes plate (40 total wells).
  - (Optional) For tube controls – Pipet 3.8  $\mu\text{L}$  of Lysis Mix A into wells A12, B12, and C12 of the new Lysis Mix A Plus Diluted Barcodes plate (one well for each tube control, see [Figure 7](#) on page 31).

- 3** Use a P10 pipette to carefully pipet the following, **gently** pipetting up and down a few times to mix and using fresh pipette tips for each well (**avoid bubbles** and **do not vortex** to avoid contamination):
  - For samples – Pipet 1.5 µL of the diluted barcodes from the Diluted Barcodes plate column by column into the 40 wells of the **new** Lysis Mix A Plus Diluted Barcodes plate.
  - (Optional) For tube controls – Pipet 1.25 µL of the diluted barcodes from wells A1, B1, and C1 of the Diluted Barcodes plate into wells A12, B12, and C12 of the **new** Lysis Mix A Plus Diluted Barcodes plate (see [Figure 7](#) on page [31](#)).
- 4** Cover the Lysis Mix A Plus Diluted Barcodes plate with PCR sealing film. Centrifuge the plate at  $\sim 3,000 \times g$  for 3 minutes (**do not vortex** to avoid contamination). Keep on ice until use.

## Reverse Transcription (RT) Reaction Mix—Mix B

- 1** Combine the following reagents in a microtube labeled “B.”

Components		Volume for samples (µL)	Volume for samples + 2 tube controls (µL)	Volume for samples + 3 tube controls (µL)
5X First-Strand Buffer (RNase-free) (C1 HT Kit <b>Module 5</b> )	●	9.5	13.6	15.65
Nuclease-Free Water (C1 HT Kit <b>Module 5</b> )	○	3.0	4.3	4.95
dNTP Mix (20 mM each) (C1 HT Kit <b>Module 5</b> )	●	2.4	3.4	3.9
RNase Inhibitor (C1 HT Kit <b>Module 5</b> )	○	1.2	1.7	1.95
Dithiothreitol (DTT) (C1 HT Kit <b>Module 5</b> )	●	1.2	1.7	1.95
Reverse Transcription Primer (C1 HT Kit Module 4)	●	2.4	3.4	3.9
Loading Reagent (C1 HT Kit Module 2)	●	0.8	1.2	1.4
Reverse Transcriptase (C1 HT Kit <b>Module 5</b> )	●	4.8	6.8	7.8
<b>Total</b>		<b>25.3</b>	<b>36.1</b>	<b>41.5</b>

- 2** Gently pipet up and down a few times to mix (**do not vortex**). Avoid creating **bubbles**. Centrifuge briefly to collect contents. Keep on ice until use.

## Preamplification Mix—Mix C

- 1 Combine the following reagents in a microtube labeled “C.”

Components	Volume for samples (µL)	Volume for samples + 2 tube controls (µL)	Volume for samples + 3 tube controls (µL)
SeqAmp PCR Buffer (2X) (Takara Bio USA, Inc.)	135.0	150.0	157.5
Loading Reagent (C1 HT Kit Module 2)	4.6	5.1	5.35
Preamp Primer (C1 HT Kit Module 2)	1.4	1.5	1.75
SeqAmp DNA Polymerase (Takara Bio USA, Inc.)	5.4	6.0	6.3
<b>Total</b>	<b>146.3</b>	<b>162.6</b>	<b>170.9</b>

- 2 Gently pipet up and down a few times to mix (**do not vortex**). Avoid creating bubbles. Centrifuge briefly to collect contents. Keep on ice until use.

## Image Cells

- 1 We highly recommend that you image all of the cell capture sites in the center panel of the HT IFC (see [Figure 8](#)) using a microscope compatible with IFCs, and then perform quality control (scoring) on the cells using an established protocol for your cell type(s).

Medium-cell HT IFC

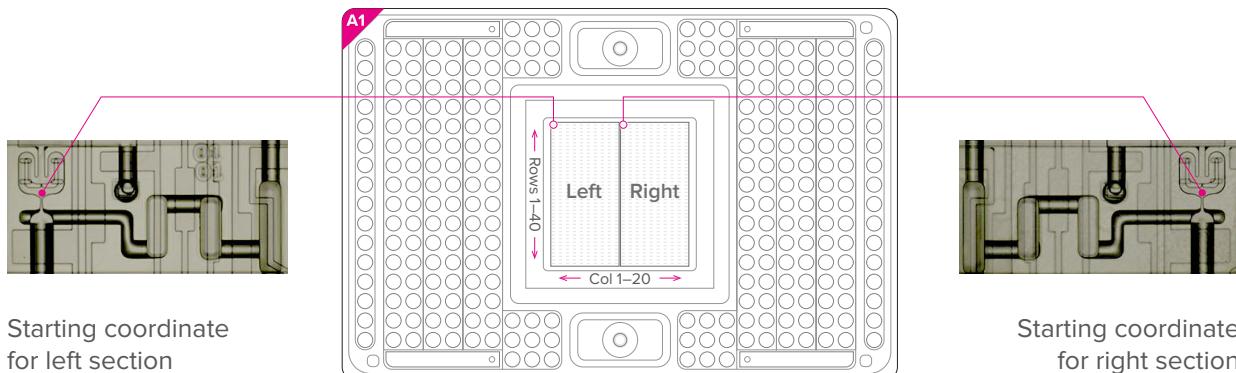


Figure 8. Starting coordinates for each section of the HT IFC.

While the vast majority of cells will be in the capture site (as shown in [Figure 9](#)), cells may occasionally be outside the capture site but still within the capture chamber where chemistry will occur. Ensure that you are imaging the larger outlined area in [Figure 9](#) to visualize all possible captured cells.

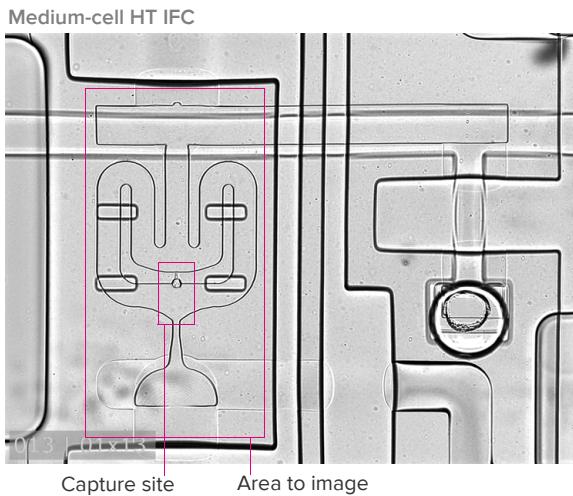


Figure 9. Cell capture area for the medium-cell HT IFC

**NOTE** See the following for additional imaging guidelines:

- Selection of a microscope – Minimum Specifications for Single-Cell Imaging (PN 100-5004).
- Viewing cell capture sites – Using a Microscope with an Automated Stage Quick Reference (PN 100-6130).
- Quality control of cells – Fluidigm Single-Cell Preparation Guide (PN 100-7697).

- 2** (Optional) If time allows, you can start the tube controls during imaging of loaded cells (see [Appendix A](#) on page [67](#) for instructions).

# Run Lysis, Reverse Transcription, and Preamplification on the C1 System

## Step 1. Remove Reagents

Remove the reagents as shown in [Figure 10](#), using a fresh pipette tip for each well.

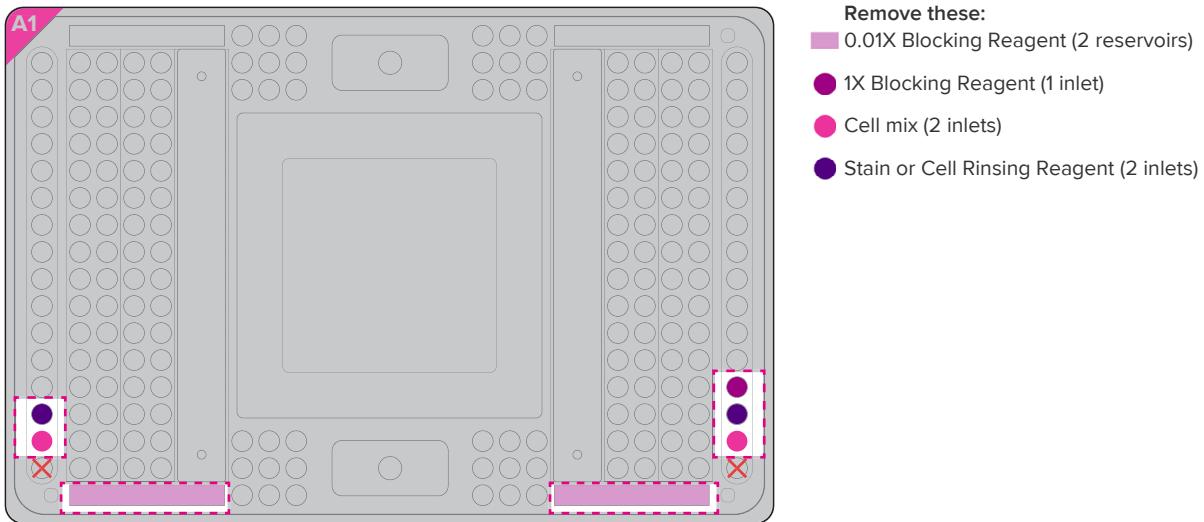


Figure 10. HT IFC remove before run pipetting map

- Use a P200 set to 180  $\mu\text{L}$  to remove the 0.01X Blocking Reagent from the 2 wash reservoirs at the bottom of the HT IFC (solid light violet rectangles ■). Some fluid can remain in the reservoir.
- Use a P20 set to 20  $\mu\text{L}$  to remove the cell mix from the 2 cell inlets (solid magenta circles ●).
- Use a P20 set to 20  $\mu\text{L}$  to remove the LIVE/DEAD staining solution or Cell Rinsing Reagent from the 2 stain inlets (solid purple circles ●).
- Use a P20 set to 20  $\mu\text{L}$  to remove the 1X Blocking Reagent from well 4, above the cell and stain inlets at the right of the HT IFC (solid dark violet circle ●).

## Step 2. Load Reagents

Before you continue, make sure to remove the reagents as shown in [Figure 10](#), and then proceed to load the reagents as shown in [Figure 11](#). Use a fresh pipette tip for each well.

**IMPORTANT** Make sure to keep the HT IFC as level as possible, and evenly distribute the reagents over the bottom surface of the reservoirs.

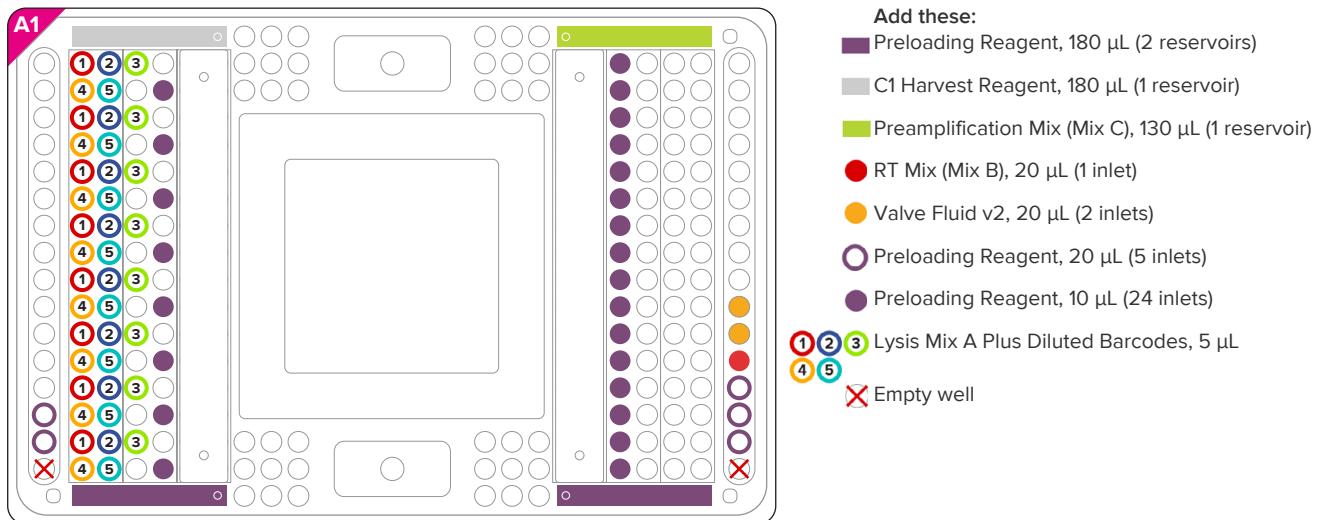


Figure 11. HT IFC lysis, RT and preamplification pipetting map

- 1 Pipet 180 µL of Preloading Reagent into each of the 2 bottom wash reservoirs (solid purple rectangles ■).
- 2 Pipet 180 µL of C1 Harvest Reagent into the top left harvest reservoir (solid gray rectangle ■).
- 3 Pipet 130 µL of Preamplification Mix (Mix C, see page [34](#)) into the top right reservoir (solid lime rectangle ■).
- 4 Pipet 20 µL of Preloading Reagent into each of 5 wells (outlined purple circle ○).
- 5 Pipet 20 µL of RT Mix B (see page [33](#)) into the RT well (solid red circle ●).
- 6 Pipet 20 µL of Valve Fluid v2 into each of 2 wells (solid gold circles ○).
- 7 Aliquot 35 µL of Preloading Reagent into each tube of an 8-tube strip, then use a multichannel pipette to pipet 10 µL of Preloading Reagent into each of the 24 inlets (solid purple circles ●).

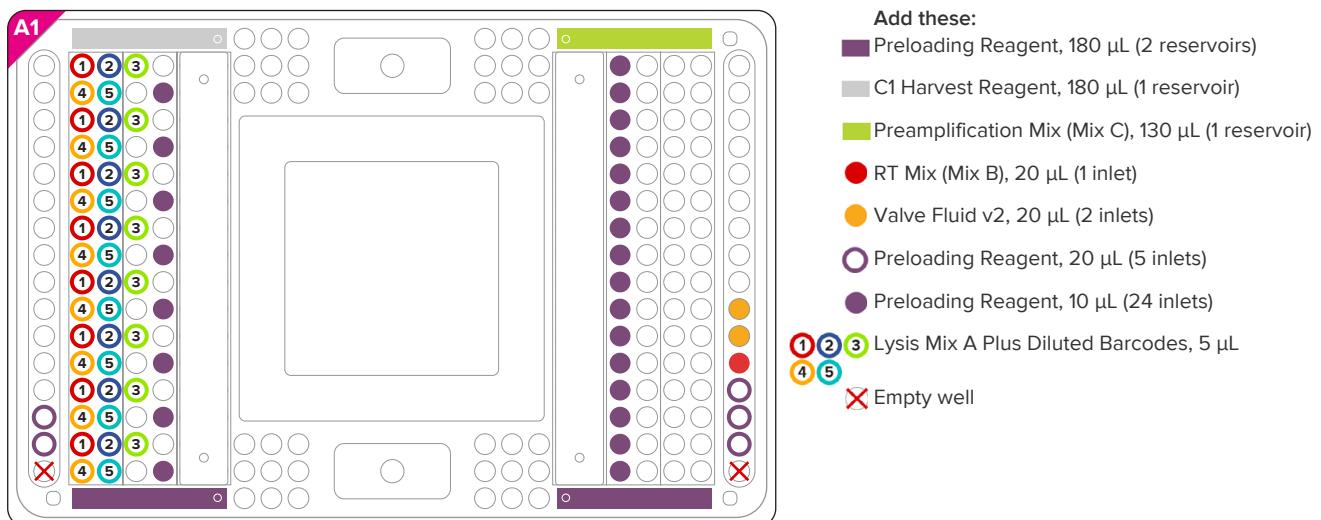


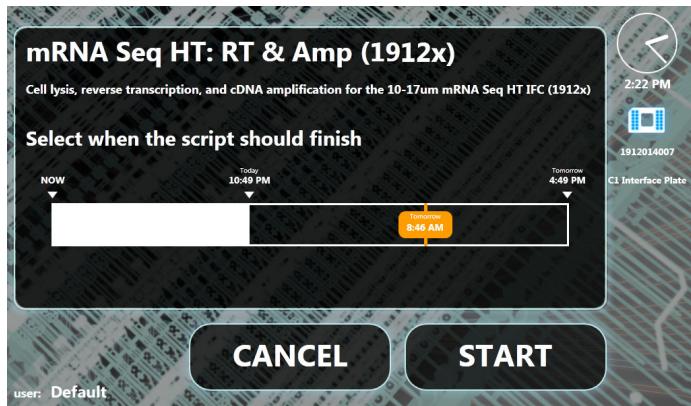
Figure 11 HT IFC lysis, RT and preamplification pipetting map [continued from page 37]

- Use a multichannel pipette with fresh pipette tips for each column to very carefully transfer, column by column, 5 µL of the Lysis Mix A containing diluted barcodes (see page 31) into each of the corresponding wells marked 1–5 in Figure 11 (①②③④⑤). **Avoid creating bubbles. Make sure to transfer the barcodes in the correct order. To avoid cross-contamination, do not allow a pipette tip to touch another well.**

**IMPORTANT** For proper demultiplexing to occur, it is critical to avoid cross-contamination. If a pipette tip touches another well, make sure to note the affected wells for use during data analysis.

- Place the HT IFC into the C1 system and immediately run the **mRNA Seq HT: RT & Amp (1912x)** script. Approximate run time: ~8.5 hours (see Appendix A for the thermal cycling protocol).

**STOPPING POINT** The script may be run overnight with a user-defined pause between PCR and harvest functions. Slide the orange box (end time) to the desired time to program the protocol to harvest at a convenient time. For example, the harvest function could be programmed to next morning.



**NOTE** To abort the script, tap **ABORT**. Do not tap ABORT unless you need to stop the experiment, as the HT IFC will no longer be usable and you must start a new experiment with a new IFC.

- 10** Make sure to store any required reagents or optional tube controls as directed elsewhere in this protocol.

## (Optional) Start the Tube Control

If you are running tube controls, see [Appendix A](#) on page [67](#) for instructions. We recommend that you start to run the tube control reaction after you start the mRNA Seq HT: RT & Amp (1912x) script.

**NOTE** If time allows, you can start the tube controls during imaging of loaded cells (see page [34](#) for imaging instructions).

## Harvest and Clean up off the C1 System

### Retrieve the Reagents for cDNA Harvest

Retrieve the following reagents (see page 14).

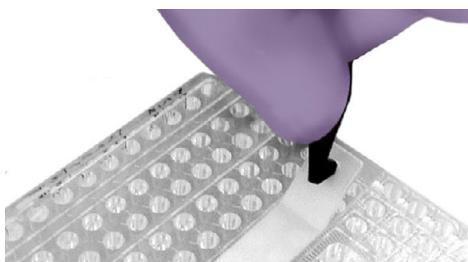
Required Reagent	Preparation	Source
Agencourt AMPure® XP	Remove from 4 °C and equilibrate to room temperature in a DNA-free hood	Beckman Coulter
DNA Suspension Reagent	Keep at room temperature	Teknova
Ethanol, 200 proof, anhydrous	Keep at room temperature	Major laboratory supplier (MLS)

### Transfer and Dilute Harvest Amplicons

- When the mRNA Seq HT: RT & Amp (1912x) script has finished, tap **EJECT** to remove the HT IFC from the instrument.

**NOTE** The HT IFC may remain in the C1 system for up to 1 hour after harvest before you remove products from their inlets.

- Transfer the HT IFC to a post-PCR lab environment.
- Label a new 96-well PCR plate “Harvest Cleanup Plate 1”.
- Aliquot 150 µL of DNA Suspension Buffer into each tube of an 8-tube strip, and then use a multichannel pipette to pipet 45 µL of buffer into the first 3 columns of the plate.
- Carefully pull back the tape covering the harvesting inlets on the left and right side of the HT IFC using the plastic removal tool:



- 6** Using a P20 multichannel pipette set to 6 µL, transfer the harvested amplicons from each column of HT IFC inlets and into the corresponding wells of the harvest plate (see [Figure 12](#)) containing DNA Suspension Buffer, for a total volume of ~50 µL in each well. The exact volume harvested from each well may vary. The expected volume is 3–6 µL.

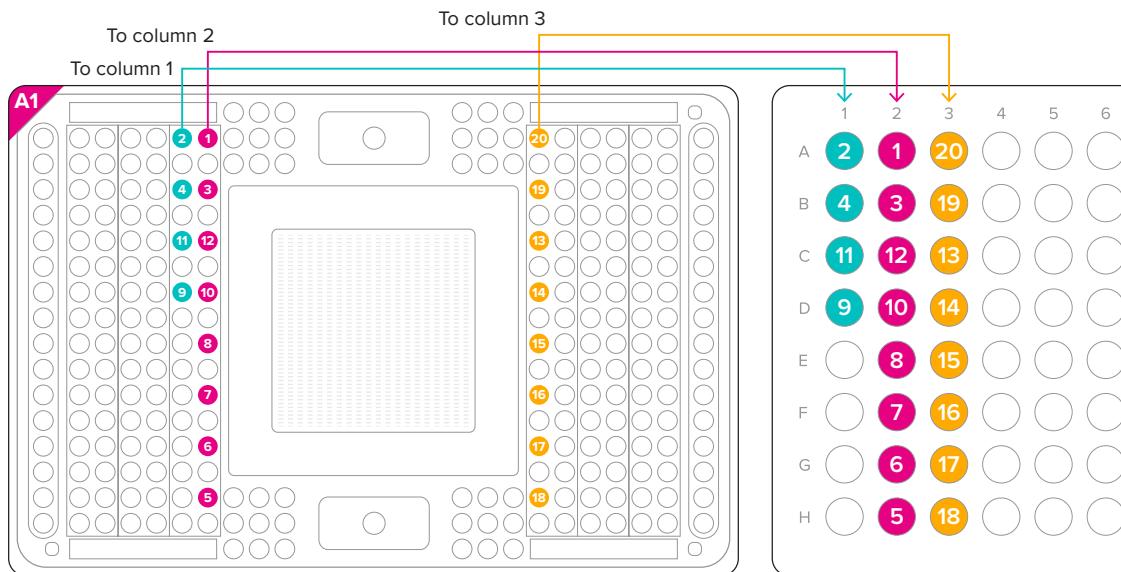


Figure 12. HT IFC harvest pipetting map (numbers refer to HT IFC column position)

- 7** (Optional) If performing a tube control, transfer 5 µL of the product from the tube control preamplification reactions (see [Process the Tube Controls with Harvest Samples on page 71](#)) into the recommended wells of the 96-well harvest plate (see [Figure 13](#)), then continue with the protocol.

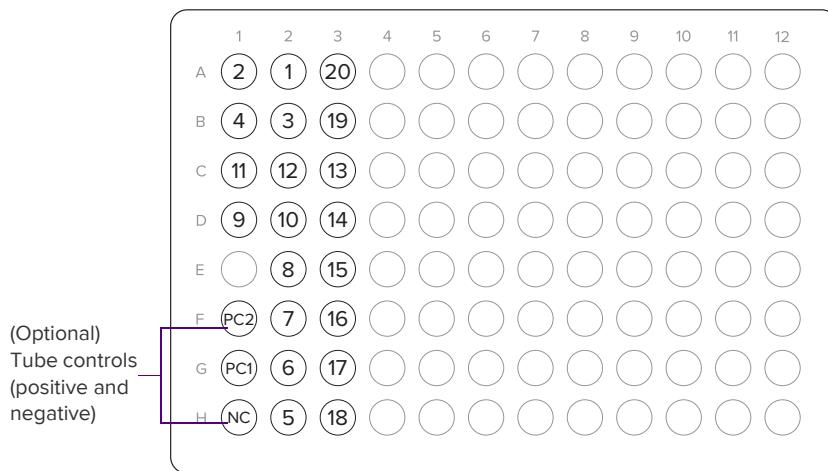


Figure 13. HT IFC column attribution to harvest plate position

**STOPPING POINT** You can store the harvest plate overnight at 4 °C or immediately proceed to cleanup.

## Clean Up Harvest Samples

### Before You Begin

Before proceeding, see the Agencourt AMPure XP PCR Purification Instructions for Use Guide (PN B37419AA) for further information and troubleshooting tips.

#### SPECIAL HANDLING FOR MAGNETIC BEADS

- Make sure to test your PCR plate and magnet for 96-well PCR plates for compatibility before use (see page 14 for recommendations). Depending on your plate/magnet combination, beads may be captured in a different area of the well than is shown in this protocol.
- Vortex the beads immediately before use and pipet carefully to ensure proper bead:sample ratios. After vortexing, the bead suspension should appear homogeneous and uniform in color. Expel any beads left in the pipette tip by pipetting the suspension up and down 5–10 times.
- Samples may be located in the supernatant or on the beads. When on the magnet, make sure the supernatant is clear and do not disturb the beads when pipetting. Change tips between samples to avoid contamination.
- Do not over dry the beads as this may decrease elution efficiency. (If over dried, beads look cracked.)

#### SPECIAL HANDLING FOR ETHANOL

Always prepare fresh 75% ethanol (EtOH) from absolute EtOH immediately before use in wash steps, and make sure to tightly close all EtOH containers when not in use. (EtOH can absorb water over time leading to a lower concentration.)

## First Cleanup

- 1 Prepare ~20 mL of 75% EtOH by measuring each component separately and mixing immediately before use. **Do not top off.**

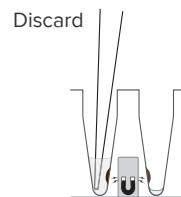
Reagent	Volume (mL)
PCR-certified water	5
EtOH (200 proof, anhydrous)	15

- 2 Warm AMPure XP beads up to room temperature and vortex for 1 minute immediately before use.
- 3 Aliquot 160  $\mu$ L of beads into each tube of an 8-tube strip, and then use a multichannel pipette to pipet 45  $\mu$ L of beads to each harvest sample (for a total volume of ~95  $\mu$ L in each well), mixing well by pipetting up and down 5–10 times and changing tips between each sample.

**IMPORTANT** Ensure beads are completely mixed and do not adhere to the side of the wells.

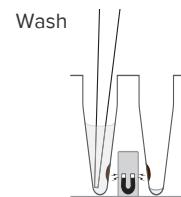
- 4 Incubate the bead mix at room temperature for 5 minutes.
- 5 Mix again by pipetting up and down 5–10 times, changing tips between each sample. Incubate again at room temperature for 5 minutes.
- 6 Place the plate on the appropriate magnet (see page 15) until the solution is clear (approximately 3–5 minutes).

- 7 Keep the plate on the magnet and use a P200 pipette set to 85  $\mu$ L to slowly and carefully remove and discard the **supernatant** from each well without disturbing the beads. We recommend that you leave ~10  $\mu$ L of the supernatant behind.



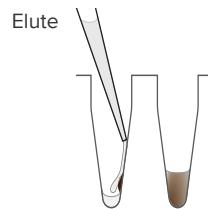
**IMPORTANT** The harvest samples are on the beads. Discard the supernatant **only**. Visually check for captured beads.

- 8 Keeping the plate on the magnet: use a multichannel pipette with fresh tips for each sample to slowly add 180  $\mu$ L of freshly prepared 75% EtOH to each well (**do not mix**), incubate at room temperature for 30 seconds, and then use a P200 pipette to remove the EtOH without disturbing the beads.



- 9 Repeat step 8.
- 10 Keep the plate on the magnet and use a P20 pipette set to 20  $\mu$ L to remove any remaining traces of EtOH without disturbing the beads. Excess carryover of EtOH may inhibit downstream reactions.

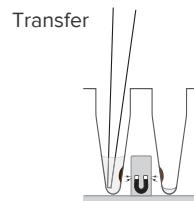
- 11 Remove the plate from the magnet and allow the beads to air-dry on bench for approximately 3–5 minutes. Visually check the beads frequently to **avoid over-drying**.
- 12 Aliquot 115 µL of Teknova DNA Suspension Buffer into each tube of an 8-tube strip, and then use a multichannel pipette to elute the samples by adding 32 µL of buffer to each well, mixing well by pipetting up and down 5–10 times until all the beads are in suspension and changing tips between each sample.



**NOTE** If the beads are over-dry and do not easily go into suspension, continue to pipet up and down until mixed.

- 13 Incubate at room temperature for 2 minutes.
- 14 Mix again by pipetting up and down 5–10 times, changing tips between each sample. Incubate again at room temperature for 2 minutes.
- 15 Place the plate on the magnet until the solution is clear (approximately 3–5 minutes).
- 16 Label a new 96-well PCR plate “Harvest Cleanup Plate 2.”
- 17 Slowly and carefully transfer 30 µL of the **supernatant** from each well to the new plate without disturbing the beads.

**IMPORTANT** The harvest samples are in the supernatant.

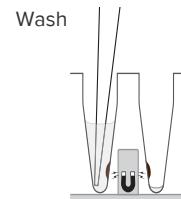
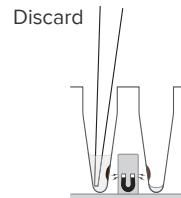


## Second Cleanup

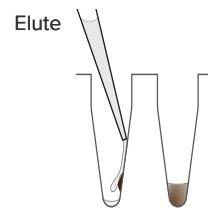
- 1 Vortex the beads for 1 minute immediately before use.
- 2 Aliquot 100 µL of beads into each tube of an 8-tube strip, and then use a multichannel pipette to pipet 27 µL of beads to each sample in the Harvest Cleanup Plate 2 (for a total volume of ~57 µL in each well), mixing well by pipetting up and down 5–10 times and changing tips between each sample.

**IMPORTANT** Ensure beads are completely mixed and do not adhere to the side of the wells.
- 3 Incubate the bead mix at room temperature for 5 minutes.
- 4 Mix again by pipetting up and down 5–10 times, changing tips between each sample. Incubate again at room temperature for 5 minutes.
- 5 Place the plate on the magnet until the solution is clear (approximately 3–5 minutes).
- 6 Keep the plate on the magnet and use a P200 pipette set to 47 µL to slowly and carefully remove and discard the **supernatant** from each well without disturbing the beads. We recommend that you leave ~10 µL of the supernatant behind.

**IMPORTANT** The harvest samples are on the beads. Discard the supernatant **only**. Visually check for captured beads.
- 7 Keeping the plate on the magnet: use a multichannel pipette with fresh tips for each sample to slowly add 180 µL of freshly prepared 75% EtOH to each well (**do not mix**), incubate at room temperature for 30 seconds, and then use a P200 pipette to remove the EtOH without disturbing the beads.
- 8 Repeat step 7.
- 9 Keep the plate on the magnet and use a P20 pipette set to 20 µL to remove any remaining traces of EtOH without disturbing the beads. Excess carryover of EtOH may inhibit downstream reactions.
- 10 Remove the plate from the magnet and allow the beads to air-dry on bench for approximately 3–5 minutes. Visually check the beads frequently to **avoid over-drying**.

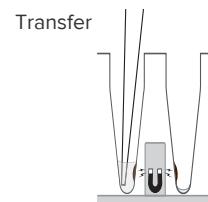


- 11** Aliquot 40 µL of DNA Suspension Buffer into each tube of an 8-tube strip, and then use a multichannel pipette to elute the samples by adding 11 µL of buffer to the beads on the side of each well, mixing well by pipetting up and down 5–10 times until all the beads are in suspension and changing tips between each sample.



**IMPORTANT** Make sure the beads are completely submerged and in suspension. For best results, align the pipette tip to the top of the bead pellet, and then slowly release the 11 µL of buffer until all of the beads are fully submerged and they drop to the bottom of the well. You can place the plate on a light color surface to easily visualize the beads. If the beads are over-dry and do not easily go into suspension, continue to pipet up and down until mixed.

- 12** Incubate at room temperature for 2 minutes.
- 13** Mix again by pipetting up and down 5–10 times, changing tips between each sample. Incubate again at room temperature for 2 minutes.
- 14** Place the plate on the magnet until the solution is clear (approximately 3–5 minutes).
- 15** Label a new 96-well PCR plate “Harvest Cleanup Plate FINAL.”
- 16** Use a single-channel pipette to slowly and carefully transfer 9 µL of the **supernatant** from each well to the new plate without disturbing the beads.
- IMPORTANT** The harvest samples are in the supernatant.
- STOPPING POINT** The samples are now ready for library preparation for sequencing (see [Prepare Next Generation Sequencing Libraries](#) on page 47). You can store the harvest amplicons overnight at 4 °C, and then proceed to [Quantify and Dilute Harvest Amplicons](#) on page 49.



# Prepare Next Generation Sequencing Libraries

**IMPORTANT** To ensure reliable results, see [Before You Begin](#) on page 42.

## Introduction

Perform the modified Illumina Nextera XT DNA library preparation protocol for single-cell mRNA sequencing on MiSeq, HiSeq®, or NextSeq systems using cDNA acquired from the HT IFC.

**IMPORTANT** The Illumina Nextera XT DNA Library Preparation Guide provides detailed instructions for next generation sequencing (NGS) library preparation; however, modifications have been made in order to adapt the Nextera XT chemistry to the single-cell mRNA sequencing application. We highly recommend that you carefully read the Nextera XT DNA Library Preparation Guide to familiarize yourself with the basic concepts and handling instructions before proceeding with this modified protocol.

**NOTE** From here, each diluted column harvest from the HT IFC is referred to as a “sample.”

## References

- Illumina Nextera XT DNA Library Preparation Guide
- Agencourt AMPure XP PCR Purification Instructions for Use Guide
- Agilent 2100 Bioanalyzer 2100 Expert User’s Guide
- Qubit 3.0 Fluorometer User Guide

# Retrieve the Reagents for Library Preparation

Retrieve the following reagents:

Required Reagents	Preparation	Source
C1 Harvest Reagent	Remove from $-20^{\circ}\text{C}$ and thaw to room temperature in a DNA-free hood	C1 HT Kit, Module 2
Enrichment Primer		
Nextera XT Index Primers (orange caps): <ul style="list-style-type: none"><li>• <b>Set A:</b> N701–N707, N710–N712, and N714–N715</li><li>• <b>Set B:</b> N716, N718–N724, and N726–N729</li></ul>	Remove from $-20^{\circ}\text{C}$ and thaw to room temperature in a DNA-free hood	Nextera XT Index Kit v2
<b>NOTE</b> Only 20 of the 24 total indices are required for use with samples (see page 53).		
Amplicon Tagment Mix	Remove from $-20^{\circ}\text{C}$ , thaw on ice, and keep on ice	Nextera XT Kit, Box 1
Tagment DNA Buffer		
Nextera PCR Master Mix (NPM)		
Neutralize Tagment (NT) Buffer	Remove from $4^{\circ}\text{C}$ and equilibrate to room temperature in a DNA-free hood	Nextera XT Kit, Box 2
Agencourt AMPure® XP	Remove from $4^{\circ}\text{C}$ and equilibrate to room temperature in a DNA-free hood	Beckman Coulter
Qubit dsDNA HS Assay Kit	Remove from $4^{\circ}\text{C}$ and equilibrate to room temperature in a DNA-free hood	Thermo Fisher Scientific
DNA Suspension Buffer	Keep at room temperature	Teknova
Ethanol, 200 proof, anhydrous	Keep at room temperature	Major laboratory supplier (MLS)
Recommended Reagents	Preparation	Source
High Sensitivity DNA Reagents	Remove from $4^{\circ}\text{C}$ and equilibrate to room temperature in a DNA-free hood	Agilent Technologies
PhiX Control v3	Remove from $-20^{\circ}\text{C}$ , thaw on ice, and keep on ice	Illumina

# Quantify and Dilute Harvest Amplicons

cDNA concentrations yielded from the C1 system may vary with cell types and cell treatments. Library yield and size distribution also vary with input cDNA/DNA concentrations. To minimize library prep variation and to achieve high library quality, carefully determine the harvest concentration and dilution.

We recommend that you determine the concentration of all column pool samples harvested from the HT IFC (and optional tube controls, if desired) on the Qubit 3.0 Fluorometer, and optionally determine the cDNA quality and size range for a subset of samples on the Agilent Bioanalyzer.

**NOTE** To maximize workflow efficiency, you can run the samples on the Qubit Fluorometer while the Agilent Bioanalyzer is running.

## Before You Begin

For best results, briefly centrifuge the “Harvest Cleanup Plate FINAL” (see page 46) and then place the plate on the appropriate magnet to ensure the solution is clear of any beads before pipetting samples from the plate.

## Determine the Concentration of All Samples

- 1 With the “Harvest Cleanup Plate FINAL” on the magnet, pipet a **minimum sample volume of 1 µL** into the Qubit dsDNA HS Assay for use on the Qubit Fluorometer. [See the Qubit 3.0 Fluorometer User Guide (Thermo Fisher Scientific PN MAN0010866) for instructions.]
- 2 Determine the effective cDNA concentration of each sample and document the results in the C1 HT IFC Workbook (Fluidigm PN 101-5976).

## Determine the cDNA Yield and Quality for a Subset of Samples

- 1 We recommend that you run at least one Agilent High Sensitivity (HS) DNA Chip with the Agilent Bioanalyzer. Select 11 samples in the “Harvest Cleanup Plate FINAL” to quantify and make sure to document your selections in the C1 HT IFC Workbook.
- 2 With the “Harvest Cleanup Plate FINAL” on the magnet, pipet **1 µL of each selected sample** into the Agilent HS DNA Chip. [See the Agilent 2100 Bioanalyzer 2100 Expert User’s Guide (Agilent Technologies PN G2946-90004) for instructions.]
- 3 For each sample run on the Agilent HS DNA Chip, assess the cDNA quality and verify the cDNA size range is within the estimate of ~200–9,000 bp (see **Figure 15** on page 75).

### Dilute the Samples

- 1 Label a new plate “Diluted Libraries (0.2 ng/µL)”.
- 2 With the “Harvest Cleanup Plate FINAL” on the magnet, pipet **at least 2 µL** of each harvested sample in the appropriate volume of C1 Harvest Reagent to achieve a final concentration of 0.2 ng/µL (based on the effective concentration in the C1 HT IFC Workbook), and pipet up and down 5–10 times to mix (**do not vortex**).

**STOPPING POINT** The samples are now ready for fragmentation (see [Perform Tagmentation](#) on page [51](#)). Keep the Diluted Libraries plate on ice until ready to use. Diluted libraries can also be stored overnight at 4 °C or long term at –20 °C.

# Perform Tagmentation

**NOTE** If you are running the optional tube controls (see [Appendix A](#) on page 67), prepare enough extra volume in the following procedures (plus 25% overage) for the number of tube controls you are running.

## Prepare cDNA for Tagmentation

**IMPORTANT** Warm Tagment DNA Buffer and Neutralize Tagment (NT) Buffer to room temperature. Visually inspect NT Buffer to ensure there is no precipitate. If there is precipitate, vortex until all particulates are resuspended.

- 1 After thawing, ensure all reagents are adequately mixed by gently inverting the tubes 3–5 times, followed by centrifuging the tubes briefly to collect the contents.

**NOTE** If you stored the Diluted Libraries plate at –20 °C, thaw the plate on ice, and then vortex at medium speed for 20 seconds. Centrifuge at ~ 3,000 × g for 3 minutes to remove bubbles.

- 2 Label a new 96-well PCR plate “Library Prep” and place it on ice.
- 3 Set up the following thermal cycling protocol, and ensure that the thermal cycler lid is heated during the incubation:

Temperature	Time
55 °C	Preheat and pause
55 °C	10 min
10 °C	Hold

- 4 In a 0.5 mL microtube on ice, combine the components of the tagmentation pre-mix. You will need enough pre-mix for each sample you wish to sequence, plus 25% overage.

Reagent	Volume per Sample (μL)	Volume for 20 Samples (plus 25% overage; μL)	Volume for 20 Samples and 2 Tube Controls (plus 25% overage; μL)	Volume for 20 Samples and 3 Tube Controls (plus 25% overage; μL)
<b>PRE-MIX</b>				
Tagment DNA Buffer	2.5	62.5	68.75	71.9
Amplicon Tagment Mix	1.25	31.25	34.4	35.9
Diluted Sample (0.2 ng/μL)	1.25	—	—	—
<b>Total</b>	<b>5.0</b>	<b>—</b>	<b>—</b>	<b>—</b>

- 5 Vortex at low speed for 20 seconds and centrifuge the tube to collect contents.

**6** Pipet 3.75  $\mu\text{L}$  of the pre-mix into each of the assigned wells of the Library Prep plate (for the 20 samples and optional tube controls). If using a multichannel pipette, aliquot 14  $\mu\text{L}$  of pre-mix into each tube of an 8-tube strip before transferring to wells.

**NOTE** We recommend that you maintain the same plate layout for tracking purposes (see [Figure 13](#) on page [41](#) and [Figure 14](#) on page [53](#)).

**7** Pipet 1.25  $\mu\text{L}$  of the diluted sample at 0.2 ng/ $\mu\text{L}$  from the diluted sample plate (see [page 50](#)) to the Library Prep plate.

**NOTE** After you transfer the samples, you can store the diluted sample plate at  $-20^\circ\text{C}$  long term, if desired.

**8** Seal the Library Prep plate and vortex at medium speed for 20 seconds. Centrifuge at  $\sim 3,000 \times g$  for 3 minutes to remove bubbles.

**9** Place the Library Prep plate in the preheated thermal cycler and skip the pause to start the protocol (see step [3](#)).

**10** While the protocol is running, aliquot equal volumes of room temperature NT Buffer into each tube of an 8-tube strip.

**NOTE** You will need 1.25  $\mu\text{L}$  of NT Buffer for each sample you wish to sequence (see step [11](#)), plus 20% overage. For 20 samples (entire HT IFC), use 4.5  $\mu\text{L}$  aliquots.

**11** Once the sample reaches  $10^\circ\text{C}$ , immediately pipet 1.25  $\mu\text{L}$  of the NT Buffer to each of the tagmented samples, and then pipette up and down 10 times to mix and quickly neutralize the samples.

**12** Seal plate and vortex at medium speed for 20 seconds. Centrifuge briefly at  $\sim 3,000 \times g$  to collect contents.

## Amplify the Tagmented cDNA

### Before You Begin

- For Index Primer selection criteria and handling instructions, carefully read the Nextera XT DNA Library Preparation Guide before proceeding to PCR amplification of the tagmented cDNA. **Do not** touch cap threads and discard and replace caps with each use.
- We recommend that you maintain the same plate layout throughout for tracking purposes (see also [Figure 13](#) on page [41](#)).

## Amplify the Tagmented DNA

- In a microtube, combine the components of the PCR pre-mix. You will need enough pre-mix for each sample you wish to sequence, plus 25% overage.

Reagent	Volume per Sample ( $\mu\text{L}$ )	Volume for 20 Samples (plus 25% overage; $\mu\text{L}$ )	Volume for 20 Samples and 2 Tube Controls (plus 25% overage; $\mu\text{L}$ )	Volume for 20 Samples and 3 Tube Controls (plus 25% overage; $\mu\text{L}$ )
Nextera PCR Master Mix (NPM)	3.75	93.75	103.1	107.8
Enrichment Primer (EP)	1.25	31.25	34.4	35.9
<b>Total</b>	<b>5.0</b>	<b>125.0</b>	<b>137.5</b>	<b>143.7</b>

- Vortex at low speed for 20 seconds and centrifuge the tube to collect contents.
- To avoid bubbles, use a single channel P20 pipette to pipet 5  $\mu\text{L}$  of PCR pre-mix into each sample well of the Library Prep plate.
- To avoid contamination, use a multichannel pipette to carefully pipet 1.25  $\mu\text{L}$  of the appropriate Index Primer from Set A or Set B to each of the 20 sample libraries on the Library Prep plate (see Figure 14). We recommend using the following indices (only 20 of the 24 total indices with orange caps are required):
  - Set A:** N701–N707, N710–N712, and N714–N715
  - Set B:** N716, and N718–N724

Each sample well should now contain a total volume of 12.5  $\mu\text{L}$ .

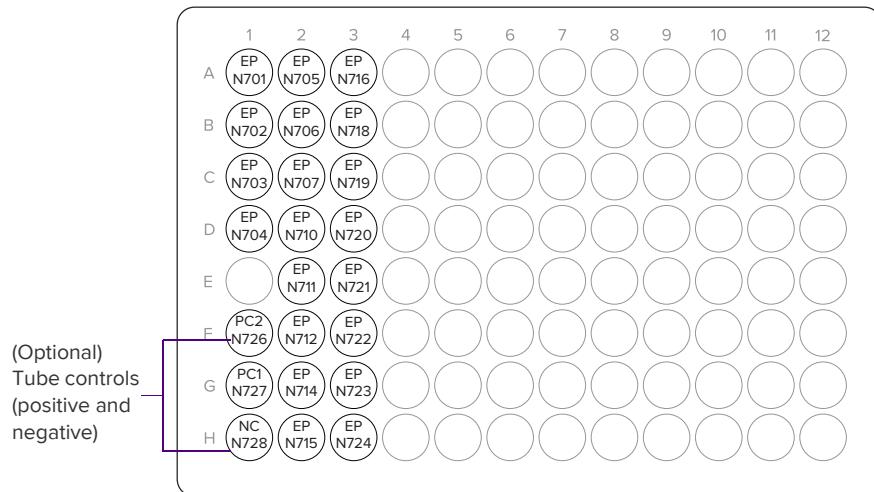


Figure 14. Index Primer pipette map and index assignment for the Library Prep plate

- (Optional) If performing a tube control (see Appendix A on page 67), select and document 2–3 of the unused indices from Set B (we recommend N726–N728) and pipet 1.25  $\mu\text{L}$  of the appropriate Index Primer to each of the recommended wells (see Figure 14 on page 53). Make sure to track the positions and indices carefully.

- 6** Seal the plate with adhesive film and vortex at medium speed for 20 seconds.  
Centrifuge at  $\sim$  3,000  $\times$  g for 3 minutes.
- 7** Place the plate into a thermal cycler and perform PCR amplification, making sure the thermal cycler lid is heated during the incubation:

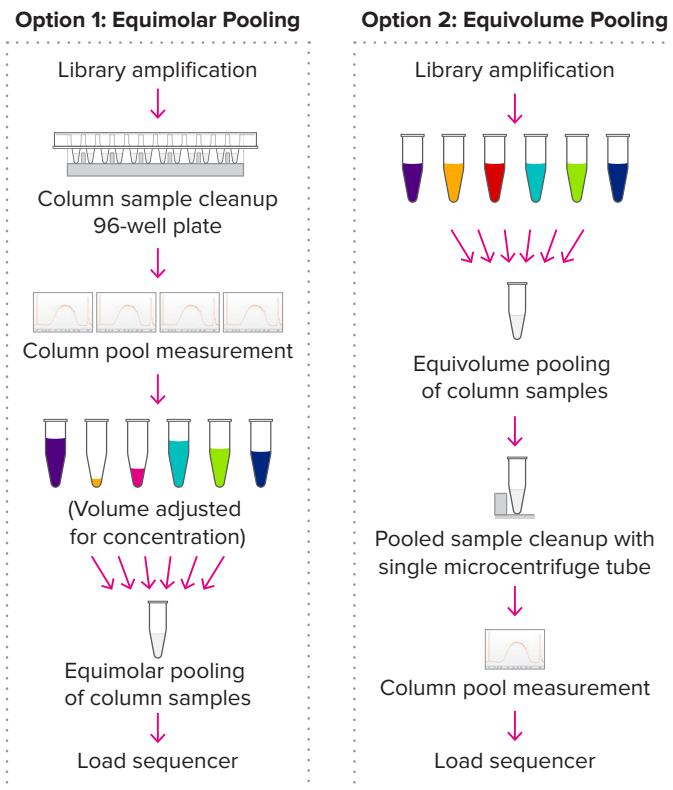
Temperature	Time	Cycles
72 °C	3 min	1
95 °C	30 sec	1
95 °C	10 sec	
55 °C	30 sec	12
72 °C	60 sec	
72 °C	5 min	1
10 °C	Hold	—

**STOPPING POINT** The PCR amplification protocol takes  $\sim$ 45 minutes to complete and may be run overnight. Amplified products can be stored overnight at 4 °C or long-term at -20 °C.

# Clean Up and Pool the Library

## Pooling Options

Choose one of the following sample pooling options:



- **Option 1. Equimolar Pooling** on page 56 – (Recommended) This option should result in more even read depth between samples (IFC columns), but requires more hands-on time. For each sample and optional tube control, you will need to perform bead purification using a magnet for 96-well PCR plates, quantification (Agilent Bioanalyzer), and then pooling based on molar concentration.
- **Option 2. Equivolume Pooling** on page 60 – Select this option if you do not need to qualify each sample individually before pooling, and to reduce hands-on time using a magnetic stand for microtubes.

## Before You Begin

- Always prepare fresh 75% ethanol (EtOH) from absolute EtOH immediately before use in wash steps, and make sure to tightly close all EtOH containers when not in use.
- If you are running the optional tube controls (see **Appendix A** on page 67), prepare enough extra volume in the following procedures (plus 25% overage) for the number of tube controls you are running.

## Option 1. Equimolar Pooling

Clean up each sample individually in a 96-well plate, quantify, and then pool in equal molar amounts into a single microtube.

### First Cleanup

- 1 Prepare ~20 mL of 75% EtOH by measuring each component separately and mixing immediately before use. **Do not** top off.

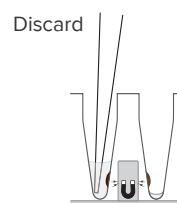
Reagent	Volume (mL)
PCR-certified water	5
EtOH (200 proof, anhydrous)	15

- 2 Keep the Library Prep plate containing the amplified samples (see page 54) at room temperature, and then use a multichannel pipette to pipet 17.5  $\mu$ L of Teknova DNA Suspension Buffer (see page 13) into each 12.5  $\mu$ L sample well, for a total volume of ~30  $\mu$ L diluted sample in each well. Optionally, you can relabel this plate “Library Cleanup Plate 1.”
- 3 Warm AMPure XP beads up to room temperature and vortex for 1 minute immediately before use.
- 4 Aliquot 80  $\mu$ L of beads into each tube of an 8-tube strip, and then use a multichannel pipette to pipet 22.5  $\mu$ L of beads to each sample (for a total volume of ~52  $\mu$ L in each well), mixing well by pipetting up and down 5–10 times and changing tips between each sample.

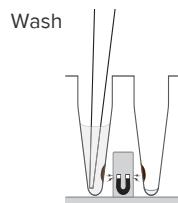
**IMPORTANT** Ensure beads are completely mixed and do not adhere to the side of the wells.

- 5 Incubate the bead mix at room temperature for 5 minutes.
- 6 Place the plate on the appropriate magnet (see page 15) until the solution is clear (approximately 3–5 minutes).
- 7 Keep the plate on the magnet and use a P200 pipette set to 42  $\mu$ L to slowly and carefully remove and discard the **supernatant** from each well without disturbing the beads. We recommend that you leave ~10  $\mu$ L of the supernatant behind.

**IMPORTANT** The samples are on the beads. Discard the supernatant **only**. Visually check for captured beads.



- 8 Keeping the plate on the magnet: use a multichannel pipette with fresh tips for each sample to slowly add 180 µL of freshly prepared 75% EtOH to each well (**do not mix**), incubate at room temperature for 30 seconds, and then use a P200 pipette to remove the EtOH without disturbing the beads.

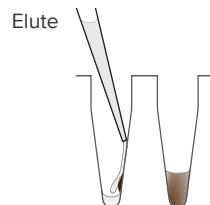


- 9 Repeat step 8.

- 10 Keep the plate on the magnet and use a P20 pipette set to 20 µL to remove any remaining traces of EtOH without disturbing the beads. Excess carryover of EtOH may inhibit downstream reactions.

- 11 Remove the plate from the magnet and allow the beads to air-dry on bench for approximately 3–5 minutes. Visually check the beads frequently to **avoid over-drying**.

- 12 Aliquot 115 µL of Teknova DNA Suspension Buffer into each tube of an 8-tube strip, and then use a multichannel pipette to elute the samples by adding 32 µL of buffer to each well, mixing well by pipetting up and down 5–10 times until all the beads are in suspension and changing tips between each sample.



**NOTE** If the beads are over-dry and do not easily go into suspension, continue to pipet up and down until mixed.

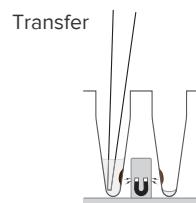
- 13 Incubate at room temperature for 2 minutes.

- 14 Mix again by pipetting up and down 5–10 times, changing tips between each sample. Incubate again at room temperature for 2 minutes.

- 15 Place the plate on the magnet until the solution is clear (approximately 3–5 minutes).

- 16 Label a new 96-well PCR plate “Library Cleanup Plate 2.”

- 17 Slowly and carefully transfer 30 µL of the **supernatant** from each well to the new plate without disturbing the beads.



**IMPORTANT** The samples are in the supernatant.

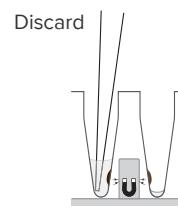
## Second Cleanup

- 1 Vortex the beads for 1 minute immediately before use.
- 2 Aliquot 80 µL of beads into each tube of an 8-tube strip, and then use a multichannel pipette to pipet 22.5 µL of beads to each sample in the Harvest Cleanup Plate 2 (for a total volume of ~52 µL in each well), mixing well by pipetting up and down 5–10 times and changing tips between each sample.

**IMPORTANT** Ensure beads are completely mixed and do not adhere to the side of the wells.

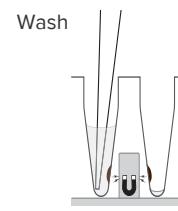
- 3 Incubate the bead mix at room temperature for 5 minutes.
- 4 Place the plate on the magnet until the solution is clear (approximately 3–5 minutes).

- 5 Keep the plate on the magnet and use a P200 pipette set to 42 µL to slowly and carefully remove and discard the **supernatant** from each well without disturbing the beads. We recommend that you leave ~10 µL of the supernatant behind.



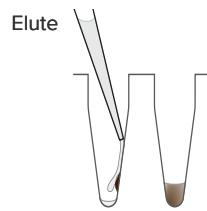
**IMPORTANT** The samples are on the beads. Discard the supernatant **only**. Visually check for captured beads.

- 6 Keeping the plate on the magnet: use a multichannel pipette with fresh tips for each sample to slowly add 180 µL of freshly prepared 75% EtOH to each well (**do not mix**), incubate at room temperature for 30 seconds, and then use a P200 pipette to remove the EtOH without disturbing the beads.



- 7 Repeat step 6.
- 8 Keep the plate on the magnet and use a P20 pipette set to 20 µL to remove any remaining traces of EtOH without disturbing the beads. Excess carryover of EtOH may inhibit downstream reactions.
- 9 Remove the plate from the magnet and allow the beads to air-dry on bench for approximately 3–5 minutes. Visually check the beads frequently to **avoid over-drying**.

- 10** Aliquot 40 µL of DNA Suspension Buffer into each tube of an 8-tube strip, and then use a multichannel pipette to elute the samples by adding 11 µL of buffer to the beads on the side of each well, mixing well by pipetting up and down 5–10 times until all the beads are in suspension and changing tips between each sample.

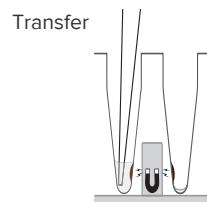


**IMPORTANT** Make sure the beads are completely submerged and in suspension. For best results, align the pipette tip to the top of the bead pellet, and then slowly release the 11 µL of buffer until all of the beads are fully submerged and they drop to the bottom of the well. You can place the plate on a light color surface to easily visualize the beads. If the beads are over-dry and do not easily go into suspension, continue to pipet up and down until mixed.

- 11** Incubate at room temperature for 2 minutes.
- 12** Mix again by pipetting up and down 5–10 times, changing tips between each sample. Incubate again at room temperature for 2 minutes.
- 13** Place the plate on the magnet until the solution is clear (approximately 3–5 minutes).
- 14** Label a new 96-well PCR plate “Library Cleanup Plate FINAL.”
- 15** Use a single-channel pipette to slowly and carefully transfer 9 µL of the **supernatant** from each well to the new plate without disturbing the beads.

**IMPORTANT** The samples are in the supernatant.

**STOPPING POINT** Keep the cleaned library at 4 °C until ready to pool, or store at –20 °C long term.



### Pool by Concentration

- 1** Place the “Library Cleanup Plate FINAL” on the magnet to ensure the solution is clear of any beads before pipetting into the Agilent HS DNA Chip.
- 2** Run 1 µL of all samples on an Agilent HS DNA Chip to check for library size distribution and quantity (see [Figure 16](#) on page [75](#)). [See the Agilent 2100 Bioanalyzer 2100 Expert User’s Guide for this step and make sure to document your results in the C1 HT IFC Workbook (Fluidigm PN 101-5976).]

**IMPORTANT** We highly recommend that you include the PhiX Control library in your sequencing run (see [page 65](#)). For best results, we recommend that you verify the PhiX library concentration (either diluted or undiluted) on an Agilent HS DNA Chip in parallel with your library samples, to verify that the PhiX concentration is correct before you combine this control with your samples in the recommended ratio for your sequencer.

- 3** Determine number of samples to be pooled based on desired sequencing depth and sequencer throughput. Keep in mind that each column pool sample from the HT IFC will include up to 40 cells.
- 4** Verify the final concentration required for your Illumina sequencer, and then adjust the volume of each library sample to equimolar amounts into a 1.5 mL low bind microtube. (See the Illumina Support Center for more information.)

**STOPPING POINT** Keep the cleaned and pooled library at 4 °C until ready to use, or store at –20 °C long term.

## Option 2. Equivolume Pooling

Pool an equal volume of each sample into a single microtube, clean up, and then quantify.

### Pool by Volume and First Cleanup

- 1** Prepare ~20 mL of 75% EtOH by measuring each component separately and mixing immediately before use. **Do not top off.**

Reagent	Volume (mL)
PCR-certified water	5
EtOH (200 proof, anhydrous)	15

- 2** Keep the amplified sample plate (see page 54) at room temperature.
- 3** Determine number of samples to be pooled based on desired sequencing depth and sequencer throughput. Keep in mind that each column pool sample from the HT IFC will include up to 40 cells.
- 4** Warm AMPure XP beads up to room temperature and vortex for 1 minute immediately before use.

**5** In a 1.5 mL low bind microtube, make the library pool as shown below:

- Pipet the appropriate volume from each sample (column 2) according to the number of samples to be pooled (column 1).
- Vortex at low speed for 20 seconds and centrifuge the tube to collect contents.
- Add the required amount of AMPure XP beads (column 4) to the pooled library, and mix well by pipetting up and down 5–10 times until all the beads are in suspension.

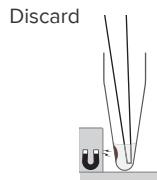
**IMPORTANT** Ensure beads are completely mixed and do not adhere to the side of the tube.

1. Number of Samples to be Pooled	2. Volume per Sample ( $\mu$ L)	3. Total Library Pool Volume ( $\mu$ L)	4. AMPure Bead Volume for Cleanup (~75% of total library pool volume; $\mu$ L)
8	4	32	24
12	4	48	36
16	2	32	24
20 (entire HT IFC)	2	40	30

**6** Incubate the bead mix at room temperature for 5 minutes.

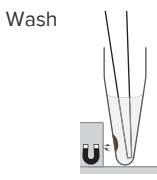
**7** Place the tube on a magnetic stand until the solution is clear (approximately 3–5 minutes).

**8** Keep the tube on the magnet and carefully remove and discard all but ~10  $\mu$ L of the **supernatant** without disturbing the beads.



**IMPORTANT** The samples are on the beads. Discard the supernatant **only**. Visually check for captured beads.

**9** Keep the tube on the magnet and slowly add 180  $\mu$ L of freshly prepared 75% EtOH (**do not mix**), incubate at room temperature for 30 seconds, and then use a P200 pipette set to 200  $\mu$ L to remove the EtOH without disturbing the beads.



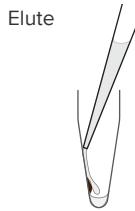
**10** Repeat step 9.

**11** Keep the tube on the magnet and use a P20 pipette set to 20  $\mu$ L to remove any remaining traces of EtOH without disturbing the beads. Excess carryover of EtOH may inhibit downstream reactions.

**12** Remove the tube from the magnet and allow the beads to air-dry at room temperature for approximately 3–5 minutes. Visually check the beads frequently to **avoid over-drying**.

- 13** Elute the samples by adding the required volume of Teknova DNA Suspension Buffer according to the number of samples pooled, mixing well by pipetting up and down 5–10 times until all the beads are in suspension:

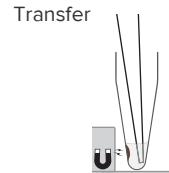
Number of Samples Pooled	DNA Suspension Buffer Volume for Elution (= original total library pool volume; $\mu\text{L}$ )
8	32
12	48
16	32
20 (entire HT IFC)	40



**NOTE** If the beads are over-dry and do not easily go into suspension, continue to pipet up and down until mixed.

- 14** Incubate for 2 minutes at room temperature.
- 15** Mix again by pipetting up and down 5–10 times. Incubate again at room temperature for 2 minutes.
- 16** Place the tube on a magnetic stand until the solution is clear (approximately 3–5 minutes).
- 17** Keep the tube on the magnet, and slowly and carefully transfer the entire volume of supernatant to another microtube without disturbing the beads.

**IMPORTANT** The samples are in the supernatant.



## Second Cleanup

- 1 Vortex the beads for 1 minute immediately before use.
- 2 Add the required volume of AMPure XP beads to the eluted samples according to the number of samples pooled, mixing well by pipetting up and down 5–10 times until all the beads are in suspension.

**IMPORTANT** Ensure beads are completely mixed and do not adhere to the side of the tube.

Number of Samples Pooled	AMPure Bead Volume for Cleanup (~75% of elution volume; µL)
8	24
12	36
16	24
20 (entire HT IFC)	30

- 3 Incubate the bead mix at room temperature for 5 minutes.
- 4 Place the tube on a magnetic stand until the solution is clear (approximately 3–5 minutes).

**NOTE** If you observe beads adhering to the sides of the tube, centrifuge the tube briefly to collect the contents. Placing the tube on the magnet for longer than 5 minutes may result in binding of unwanted products.

- 5 Keep the tube on the magnet and carefully remove and discard all but ~10 µL of the **supernatant** without disturbing the beads.

**IMPORTANT** The samples are on the beads. Discard the supernatant **only**. Visually check for captured beads.

- 6 Keep the tube on the magnet and slowly add 180 µL of freshly prepared 75% EtOH (**do not mix**), incubate at room temperature for 30 seconds on the magnetic stand, and then use a P200 pipette set to 200 µL to remove the EtOH without disturbing the beads.

- 7 Repeat step 6.
- 8 Keep the tube on the magnet and use a P20 pipette set to 20 µL to remove any remaining traces of EtOH without disturbing the beads. Excess carryover of EtOH may inhibit downstream reactions.
- 9 Remove the tube from the magnet and allow the beads to air-dry at room temperature for approximately 3–5 minutes. Visually check the beads frequently to **avoid over-drying**.



- 10** Elute the samples by adding the required volume of DNA Suspension Buffer according to the number of samples pooled, mixing well by pipetting up and down 5–10 times until all the beads are in suspension:

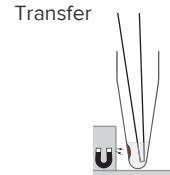
Number of Samples Pooled	Volume of DNA Suspension Buffer for Elution (~90% of original pool volume; µL)
8	28.8
12	43.2
16	28.8
20 (entire HT IFC)	36



**NOTE** If the beads are over-dry and do not easily go into suspension, continue to pipet up and down until mixed.

- 11** Incubate at room temperature for 2 minutes.
- 12** Mix again by pipetting up and down 5–10 times. Incubate again at room temperature for 2 minutes.
- 13** Place the tube on the magnetic stand until the solution is clear (approximately 3–5 minutes).

- 14** Set a P200 pipette to ~90% of the elution volume and carefully transfer the **supernatant** to another microtube without disturbing the beads. (For example, for 20 samples you will transfer 33 µL of supernatant.)



**IMPORTANT** The sample is in the supernatant.

- 15** Run 1 µL of the pooled sample in triplicate on an Agilent HS DNA Chip to check for library size distribution and quantity (see [Figure 16](#) on page [75](#)). Place the pooled sample on the magnet to ensure the solution is clear of any beads before pipetting into the Agilent HS DNA Chip. (See the Agilent 2100 Bioanalyzer 2100 Expert User’s Guide for this step.)

**IMPORTANT** We highly recommend that you include the PhiX Control library in your sequencing run (see [page 65](#)). For best results, we recommend that you verify the PhiX library concentration (either diluted or undiluted) on an Agilent HS DNA Chip in parallel with your library samples, to verify that the PhiX concentration is correct before you combine this control with your samples in the recommended ratio for your sequencer.

**STOPPING POINT** Keep the pooled and cleaned library at 4 °C until ready to use, or store at –20 °C long term.

# Requirements and Recommendations for Illumina Sequencing

## Library Concentration for Sequencing

- Base diversity can facilitate cluster calling. We highly recommend that you add the following PhiX spike-in concentrations to your samples, depending on the Illumina sequencer and version of Real-Time Analysis (RTA) software you are using.

Illumina Sequencer	PhiX Concentration (% of total sample concentration)
MiSeq	1–5%
	<b>NOTE</b> Although not required for base diversity on MiSeq, a small percentage of PhiX is recommended for estimating sequencing metrics and for troubleshooting sequencing runs.
HiSeq 2500	5%
HiSeq 3000/4000	10–20%
NextSeq®	20–40%

- See the denature and dilute libraries guide for your Illumina sequencer to determine the appropriate loading concentration for sequencing and dilute the pooled sample library and the PhiX library as needed.

**NOTE** The PhiX Control is provided at a 10 nM concentration. It may be necessary to dilute this library to ~2 nM or lower prior to combining with your pooled library. Make sure to verify the concentration of your sample library and the PhiX control library on the Agilent Bioanalyzer prior to combining (see page 59 or page 64).

- We recommend that you first combine the PhiX library with the sample library in the recommended concentration as shown above, and then denature as instructed in the denature and dilute libraries guide for your Illumina sequencer.

## Sample Naming and Sequencing Read Length

### Sample Naming Recommendations

For best results with the C1 mRNA Sequencing High Throughput Demultiplexer Script (v2.0.1 or later, see [Appendix B](#) on page [72](#)), we recommend that you follow the sequencing sample naming recommendations below, using the C1 HT IFC Workbook (PN 101-5976) as a guide.

- Begin each Sample ID in the Illumina sample sheet with “<samplename>-COL<N>,” where:
  - <samplename> = your sample name (alphanumeric, no spaces)
  - - (hyphen) = allowed separator
  - COL<N> = column number from the HT IFC (0-padded to 2 digits)

All other characters are invalid. For example, if you are interested in sequencing a sample harvested from Column 1 in the HT IFC, an entry of “HT1912123456-ControlCells-COL01” in the Sample ID field of the sample sheet is acceptable in order to generate a FASTQ file starting with the recommended information.

- If you use the Illumina Experiment Manager (IEM) software to set up your Illumina sample sheet, make sure to set the Index Reads field to 1. The number of Index Cycles is automatically set to 8 based on this selection. (See the Illumina website for more information.)

### Sequencing Read Length Recommendations

For accurate paired-end sequencing results, we recommend that you run the following number of cycles for each of the Illumina sequencer reads (see [Figure 2](#) on page [9](#)):

- **Read 1 (R1)** – Use a minimum of 26 cycles, to identify the sample based on the row barcode sequence, and for cluster identification. Increasing the number of cycles for Read 1 will not provide additional sequencing information.
- **Read 2 (R2)** – Use a minimum of 75 cycles, to generate sufficient read length for accurate sequence alignment.

# Appendix A: (Optional) Run the Tube Controls

## Introduction

The tube controls are used as positive and negative controls for the C1 Single-Cell mRNA Seq HT reagents and workflow performed off the HT IFC (see page 10). The results from the multi-cellular positive tube control can also be compared with the single-cell samples harvested from the IFC. You prepare two types of tube controls: one or more with cells (positive control or PC), and one without cells (no template control or NTC). You perform the tube control reactions (lysis, RT, and preamplification) off the IFC using the same chemistry you use to process the single cells on the IFC.

## Wash the Cells

Before use in the tube control reactions, wash the cells you reserved in the tube labeled “PC” (see step 2 on page 26) using 1X PBS or similar user-supplied wash buffer appropriate for your cell type:

- 1 Pellet the reserved cells in the tube labeled “PC”. Speeds and durations may vary. We suggest centrifuging the 1.5 mL microtube containing the reserved cells at 300  $\times g$  for 5 minutes.
- 2 Gently remove the supernatant without disturbing the cell pellet.  
**IMPORTANT** The cell pellet may be difficult to see at lower volumes or cell concentrations. Make sure to **pipet slowly and carefully** to avoid accidental transfer of cells into the supernatant.
- 3 Resuspend cells in 1 mL of user-supplied wash buffer by gently pipetting up and down at least 5 times. This is wash 1.
- 4 Repeat steps 1–2.
- 5 Resuspend cells again in 1 mL of user-supplied wash buffer by gently pipetting up and down at least 5 times. This is wash 2.
- 6 Repeat steps 1–2.

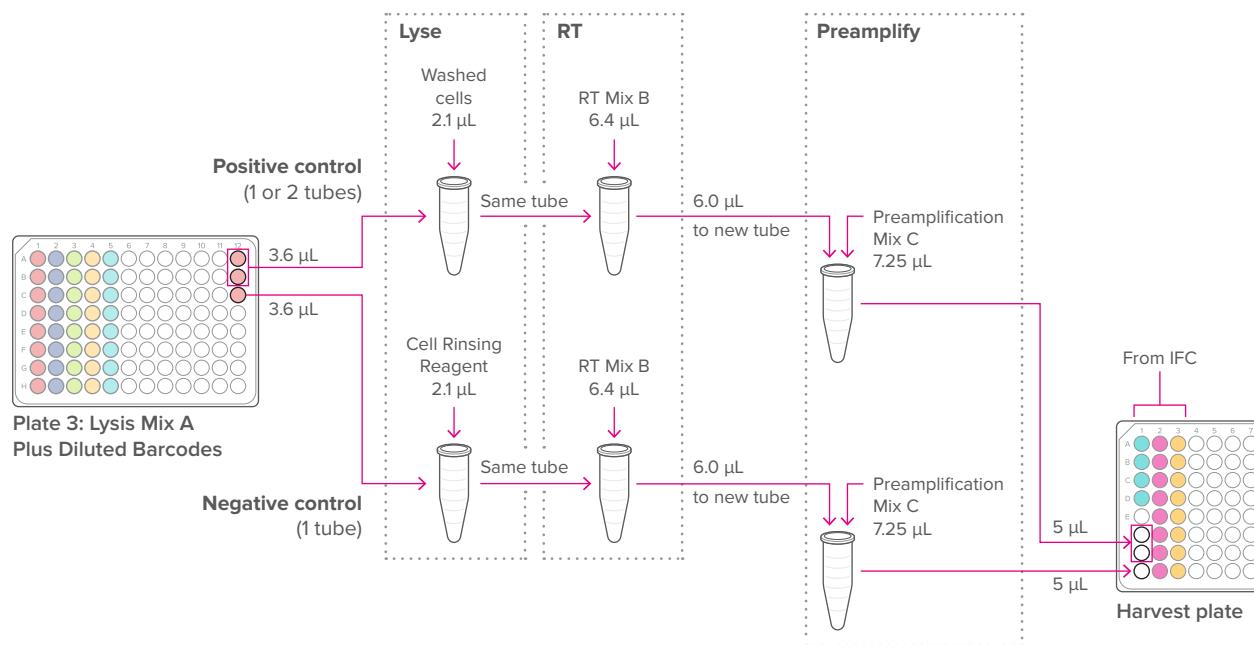
- 7** Resuspend cells in user-supplied wash buffer to approximately 50% of original volume to achieve a concentration of 250–500 cells/ $\mu$ L.

**NOTE** Cells may be counted by any preferred method. If an established cell counting protocol does not exist, we suggest using the disposable hemocytometer C-Chip by INCYTO. See [incyto.com/product/product02\\_detail.php](http://incyto.com/product/product02_detail.php) for instructions for use.

- 8** Keep the washed cells on ice until use.

## Perform the Tube Control Reactions

You can prepare two or three tube controls, either one NTC and one PC, or one NTC and two PCs (one for each section of the HT IFC).



## Lysis

Prepare the tube control reactions by combining lysis reagents and thermal cycling them.

- 1 Prepare cell lysis mix in 2 or 3 tubes of an unused 8-tube strip:

Components	NTC (no template control; $\mu\text{L}$ )	PC (positive control; $\mu\text{L}$ )*
Washed cells (see page 67)	—	2.1
Cell Rinsing Reagent	○	2.1
Lysis Mix A plus diluted barcodes (from step 3b on page 33))	3.6	3.6
<b>Total</b>	<b>5.7</b>	<b>5.7</b>

\* Include a second positive control if you load two different cell mixes into the HT IFC (see page 24).

- 2 Mix gently and centrifuge briefly to collect contents.

- 3 In a thermal cycler, run the following cell lysis protocol:

Temperature	Time
72 °C	3 min
4 °C	10 min
25 °C	1 min
4 °C	Hold

## Reverse Transcription (RT)

- 1 After the cell lysis protocol has finished, prepare the RT reaction by adding RT Mix B to the appropriate tubes containing cell lysis products (from step 3 above):

Components	NTC ( $\mu\text{L}$ )	PC ( $\mu\text{L}$ )*
Cell lysis products (from step 3 above)	5.7	5.7
RT Mix (Mix B, see page 33)	6.4	6.4
<b>Total</b>	<b>12.1</b>	<b>12.1</b>

\* Include a second positive control if you load two different cell mixes into the HT IFC (see page 24).

- 2 Mix gently and centrifuge briefly to collect contents.

- 3** In a thermal cycler, run the following RT protocol:

Temperature	Time
42 °C	120 min
4 °C	Hold

**STOPPING POINT** If you program to harvest the next day (see page 38), you can store the RT reaction and the Preamplification Mix C (see page 34) overnight at 4 °C until you are ready to run the PCR reaction.

### Preamplification (PCR)

- 1** After the RT protocol has finished, prepare the preamplification reaction by combining the RT reaction products (from step 3 above) and Preamplification Mix C in the appropriate tubes of an unused 8-tube strip:

Components	NTC ( $\mu$ L)	PC ( $\mu$ L)*
RT reaction (from step 3 above)	6.0	6.0
Preamplification Mix (Mix C, see page 34)	7.25	7.25
<b>Total</b>	<b>13.25</b>	<b>13.25</b>

\* Include a second positive control if you load two different cell mixes into the HT IFC (see page 24).

- 2** Mix gently and centrifuge briefly to collect contents, then run the following PCR protocol:

Temperature	Time	Cycles
96 °C	1 min	1
98 °C	10 sec	
59 °C	4 min	4
68 °C	6 min	
98 °C	10 sec	
59 °C	1 min	14
68 °C	4 min	
72 °C	5 min	1
4 °C	Hold	1

- 3** Centrifuge briefly to collect contents.

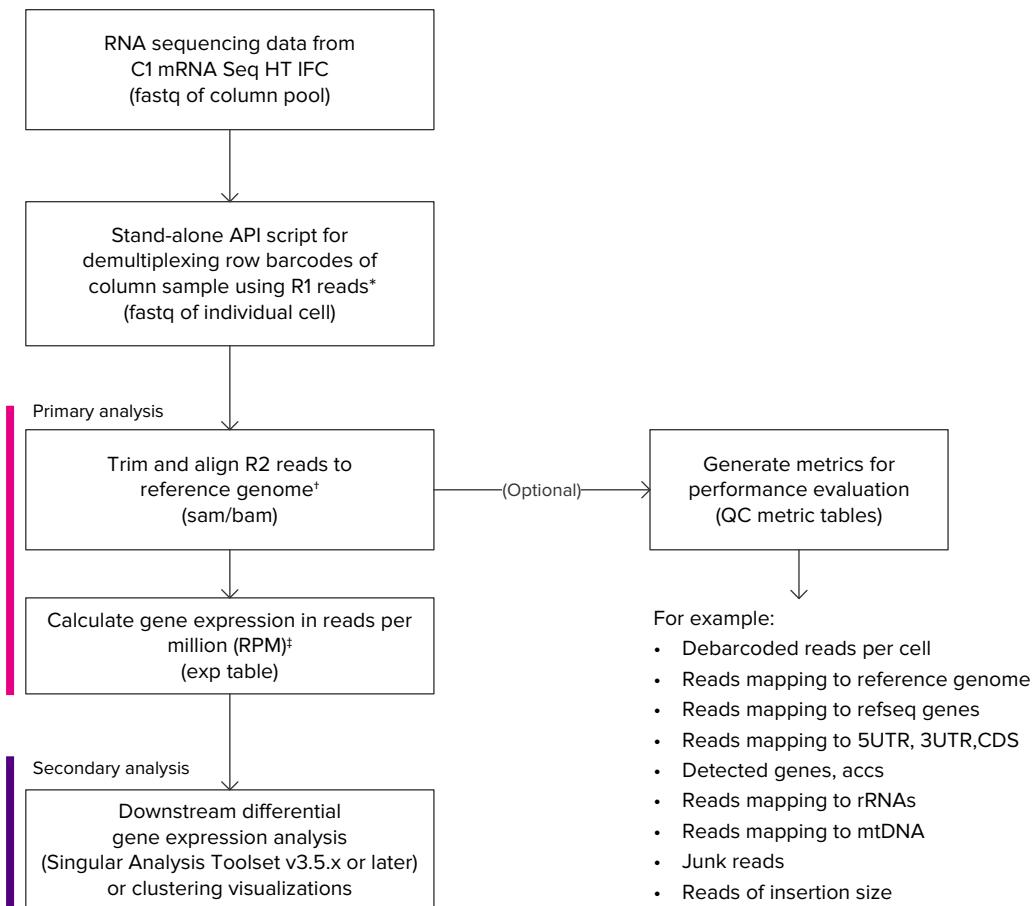
## Process the Tube Controls with Harvest Samples

- 1 Transfer the PCR products (from step 3 above) to a post-PCR room.
- 2 Vortex the prepared PCR products for 3 seconds and centrifuge briefly to collect contents.
- 3 Transfer 5  $\mu$ L of the tube control samples to the appropriate wells of the harvest plate (see [Harvest and Clean up off the C1 System](#) on page [40](#)), then continue with the protocol and process the tube control samples along with the harvested samples.

# Appendix B: Data Analysis

## Analysis Workflow

The workflow for analysis of RNA sequencing data from the C1 mRNA Seq HT IFC protocol is as follows:



Sequencing data:

\* HT Demultiplex script available from [fluidigm.com/software](http://fluidigm.com/software)

Primary analysis recommendations:

† Alignment by Tophat/rsem

‡ Gene expression calculation by Cufflink/rsem

## Stand-alone Script for Demultiplexing

Initially, only the column pool samples loaded onto the sequencer are demultiplexed from the Illumina MiSeq or HiSeq sequencer reads (R1 and R2) because the sequencer only demultiplexes Nextera indices (column pools). The C1 mRNA Sequencing High Throughput Demultiplexer Script is available for download from the Fluidigm Software products page as a Perl script application programming interface (API) for use on a Linux™ operating system.

The script allows you to automatically:

- Demultiplex the individual single-cell samples from each column using the cell barcodes on the R1 reads.
- Separate the large FASTQ file generated from each column pool into 40 pairs (R1 and R2) of FASTQ files, one pair for each row in the HT IFC.
- If running script v2.0.1 or later:
  - Combine FASTQ files for the same sample if run in multiple lanes on the sequencer.
  - Generate a demultiplex report of the number of reads for each cell sample.

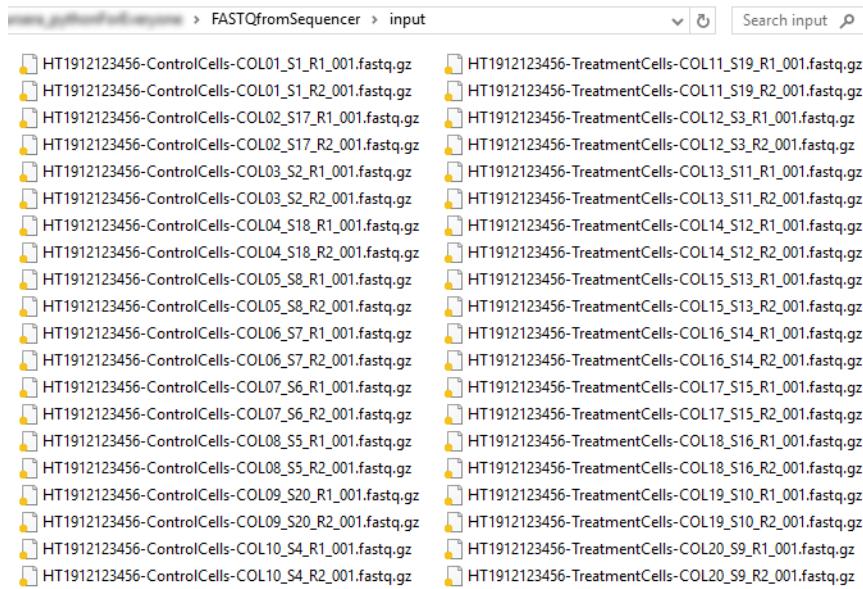
## Download and Install the Script

- 1 Go to [fluidigm.com/software](http://fluidigm.com/software) and download the “C1\_mRNA\_Seq\_HT\_Demultiplex\_Script\_[version].zip” file.
- 2 Unzip the downloaded file, then copy the “C1\_mRNA\_Seq\_HT\_Demultiplex\_Script\_[version]” folder to a server location of your choice.
- 3 Within this folder, create two new folders to contain the individual FASTQ files:
  - **input** – input directory where raw FASTQ files for each column pool are placed
  - **output** – output directory of demultiplexed (individual cell) FASTQ files for each row (this directory is empty prior to running the script for the first time)

Name	Type
input	File folder
output	File folder
mRNASEqHT_demultiplex.pl	PL File

## Run the Script

- 1** Make sure the raw FASTQ files from each column are in the “input” folder (see page 65 for naming recommendations, for example: “<samplename>-COL<N>”).



- 2** Navigate to and run the C1 mRNA Sequencing High Throughput Demultiplexer Perl script API (“mRNASeqHT\_demultiplex.pl”) with this command:

```
perl mRNASeqHT_demultiplex.pl -i input -o output
```

- 3** (v2.0.1 or later) The script demultiplexes the raw FASTQ files in the “input” folder into multiple FASTQ files, appended with “\_ROW<N>”, and saves these processed files to the “output” folder.

For example, if a raw FASTQ file is named “HT1912123456-ControlCells-COL01”, the script will output HT1912123456-ControlCells-COL01\_ROW<N> into 40 pairs of FASTQ files (where N is 0-padded to 2 digits).

**NOTE** Contact the IT or bioinformatics representative at your site if you require assistance to run this script with your analysis pipeline.

## Additional Analysis Recommendations

- Before aligning R2 reads to the reference genome, trim the polyA stretch from the 3’ end of the R2 reads.
- Use only R2 reads for the downstream analysis for end-counting in transcriptome expression, as described in the analysis workflow (see page 72).

For more information on downstream differential gene expression analysis, see the Singular Analysis Toolset User Guide (version 3.5.x or later).

# Appendix C: Library Prep Examples

## Typical Agilent Bioanalyzer Trace After Harvest

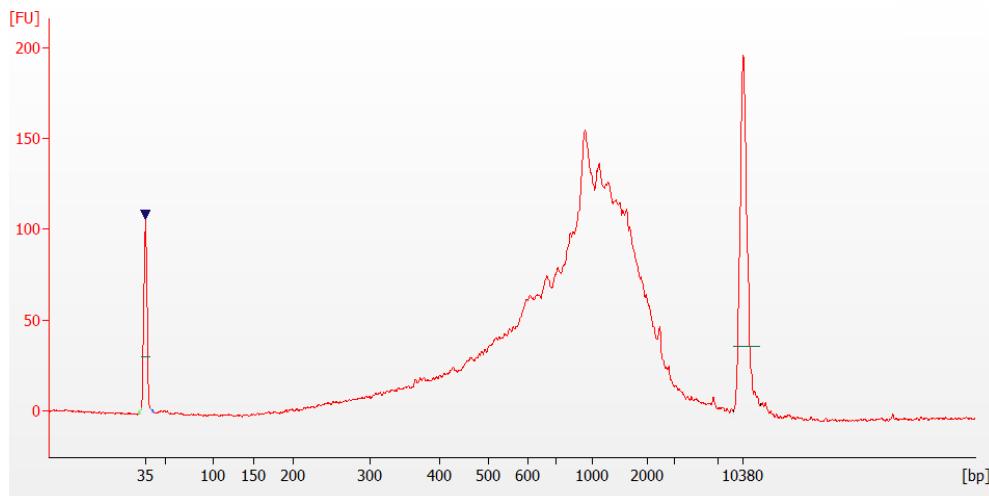


Figure 15. A typical cDNA individual column pool harvested from the HT IFC (after preamplification on the HT IFC)

## Typical Agilent Bioanalyzer Trace After Tagmentation

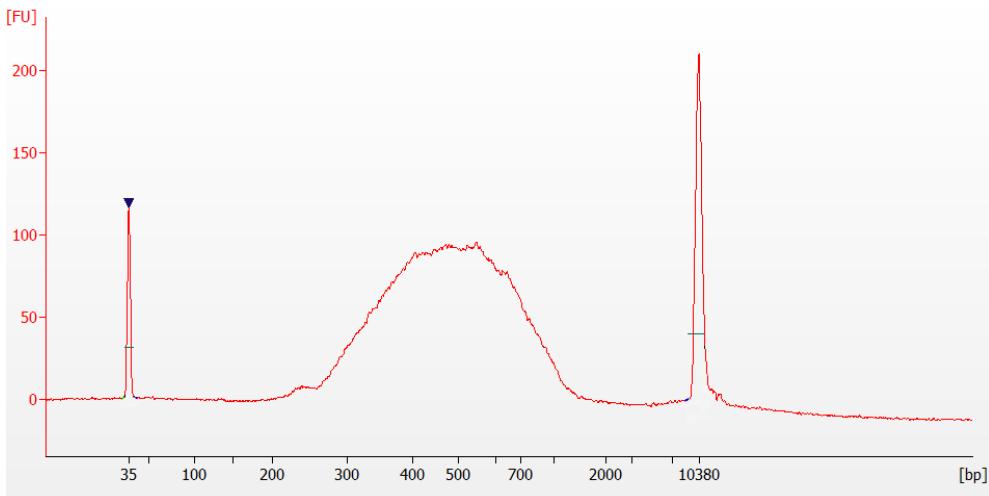


Figure 16. Typical library size distribution (after tagmentation, amplification, and purification)

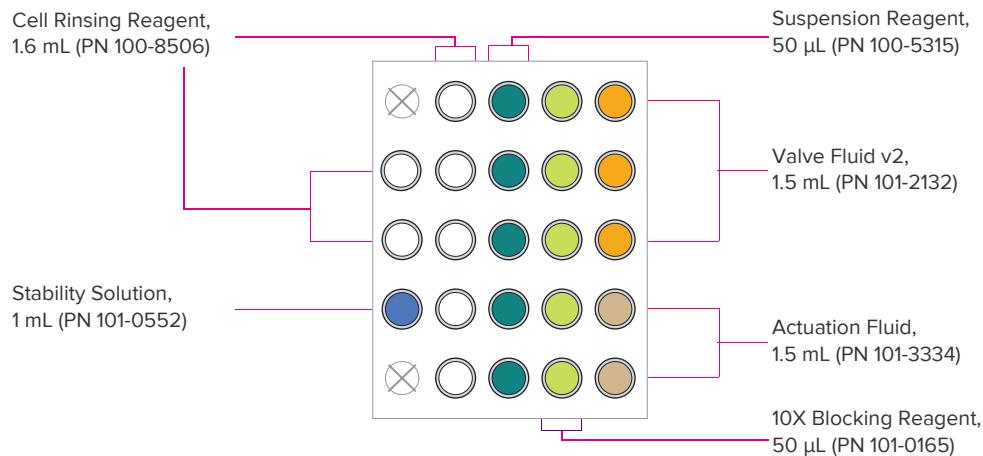
# Appendix D: C1 Single-Cell mRNA Seq HT Reagent Kit v2, PN 101-3473

## About the Kit

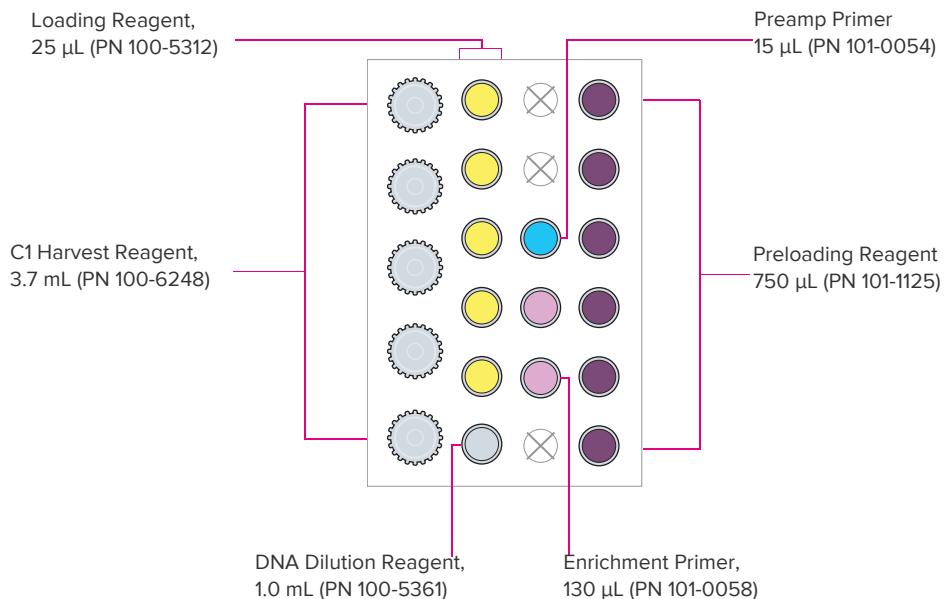
- The C1 Single-Cell mRNA Seq HT Reagent Kit v2 is shipped in five boxes: Module 1, Module 2, Module 3, Module 4, and Module 5.
- When ordering the modules from Fluidigm, use the kit part number: 101-3473.
- The modules in this kit provide the necessary reagents to run mRNA Seq HT chemistry on five C1 HT IFCs.
- For storage conditions, see [Required Reagents](#) on page 13. For usage, see page 18.

## Kit Contents

### Module 1 (PN 101-3474)



## Module 2 (PN 101-0060)



## Module 3 (PN 101-0061)

Each Cell Barcode Plate contains 40 concentrated cell (row) barcodes that are single-use only. The numbers below are the HT IFC row assignments for each well in the plate.

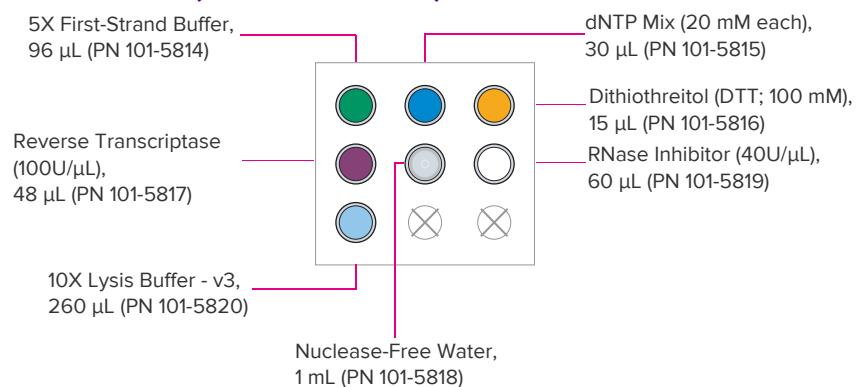
	1	2	3	4	5	6	7	8	9	10	11	12
A	2	1	3	4	5							
B	6	7	8	9	10							
C	11	12	13	14	15							
D	16	17	18	19	20							
E	23	22	21	25	24							
F	28	27	26	30	29							
G	33	32	31	35	34							
H	38	37	36	39	40							

Cell Barcode Plate, 40 barcodes (PN 101-0164)

## Module 4 (PN 101-0062)

Reverse Transcription Primer, 20 μL (PN 100-0056)

## Module 5 (PN 101-5823)



# Appendix E: HT IFC Types and Related Scripts

There is currently one C1 system-compatible HT IFC for medium single cells:

Cell Size/IFC Name and Part Numbers	Barcode	Script Names*	Description
<b>For mRNA Seq HT protocol:</b>			
<b>Medium (10–17 µm)</b> C1 IFC for mRNA Seq HT (PN 101-4981)	1912x	mRNA Seq HT: Prime (1912x)	Prime the control line and cell capture channels of the 10–17 µm mRNA Seq HT IFC (1912x)
		mRNA Seq HT: Cell Load (1912x)	Cell loading and washing without staining for the 10–17 µm mRNA Seq HT IFC (1912x)
		mRNA Seq HT: Cell Load & Stain (1912x)	Cell loading, staining, and washing for the 10–17 µm mRNA Seq HT IFC (1912x)
		mRNA Seq HT: RT & Amp (1912x)	Cell lysis, reverse transcription, and cDNA amplification for the 10–17 µm mRNA Seq HT IFC (1912x)

\* Make sure to install C1 system software v2.2.3 or later to run the latest scripts. For more information, see the C1 System Software Release Notes (PN 101-5841) and Updating the C1 System Software Quick Reference (PN 100-6217).

# Appendix F: Related Documentation

## cDNA Synthesis

- Agencourt AMPure XP PCR Purification Instructions for Use Guide (Beckman Coulter, PN B37419AA)
- Agilent 2100 Bioanalyzer 2100 Expert User's Guide (Agilent Technologies PN G2946-90004)
- ArrayControl Spots and Spikes (Thermo Fisher Scientific PN AM1781)
- C1 mRNA Sequencing DataSheet (Fluidigm PN 101-0984)
- C1 System User Guide (Fluidigm PN 100-4977)
- Fluidigm Single-Cell Preparation Guide (Fluidigm PN 100-7697)
- INCYTO Disposable Hemocytometer, [incyto.com/product/product02\\_detail.php](http://incyto.com/product/product02_detail.php)
- LIVE/DEAD Viability/Cytotoxicity Kit, for mammalian cells (Thermo Fisher Scientific PN L-3224)
- Minimum Specifications for Imaging Cells in Fluidigm Integrated Fluidic Circuits Specification Sheet (Fluidigm PN 100-5004)
- Pipetting Maps for the C1 mRNA Seq HT v2 Protocol: Quick Reference (Fluidigm PN 101-4965)
- Singular Analysis Toolset User Guide (Fluidigm PN 100-5066)
- Using a Microscope with an Automated Stage Quick Reference (Fluidigm PN 100-6130)
- C1 HT IFC Workbook (Fluidigm PN 101-5976)

## DNA Sequencing

- Illumina Nextera XT DNA Library Preparation Guide (Illumina PN 15031942)
- Qubit 3.0 Fluorometer User Guide (Thermo Fisher Scientific PN MAN0010866)

# Appendix G: Safety

## General Safety

In addition to your site-specific safety requirements, Fluidigm recommends the following general safety guidelines in all laboratory and manufacturing areas:

- Use the appropriate personal protective equipment (PPE): safety glasses, fully enclosed shoes, lab coats, and gloves, according to your laboratory safety practices.
- Know the locations of all safety equipment (fire extinguishers, spill kits, eyewashes/showers, first-aid kits, safety data sheets, etc.), emergency exit locations, and emergency/injury reporting procedures.
- Do not eat, drink, or smoke in lab areas.
- Maintain clean work areas.
- Wash hands before leaving the lab.

## Instrument Safety

For complete instrument safety information, including a full list of the symbols on the instrument, refer to the C1 System User Guide (PN 100-4977).



**WARNING** BIOHAZARD. If you are putting biohazardous material on the instrument, use appropriate personal protective equipment and adhere to Biosafety in Microbiological and Biomedical Laboratories (BMBL), a publication from the Centers for Disease Control and Prevention, and to your lab's safety protocol to limit biohazard risks. If biohazardous materials are used, properly label the equipment as a biohazard. For more information, see the BMBL guidelines at: [cdc.gov/biosafety/publications/index.htm](http://cdc.gov/biosafety/publications/index.htm).

## Chemical Safety

The responsible individuals must take the necessary precautions to ensure that the surrounding workplace is safe and that instrument operators are not exposed to hazardous levels of toxic substances. When working with any chemicals, refer to the applicable safety data sheets (SDSs) provided by the manufacturer or supplier.

## Disposal of Products

Used IFCs and reagents should be handled and disposed of in accordance with federal, state, regional, and local laws for hazardous waste management and disposal.



For technical support visit [fluidigm.com/support](http://fluidigm.com/support)