Pipetting Maps for the C1 mRNA Seq HT v2 Protocol

Before You Begin

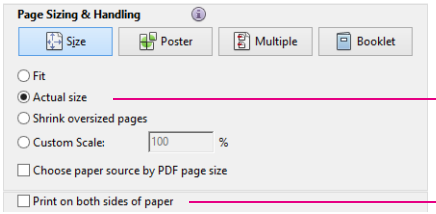
Before using the maps and for detailed instructions on preparing the C1™ mRNA Seq HT v2 chemistry, see the Generate cDNA Libraries with the C1 Single-Cell mRNA Seq HT IFC and Reagent Kit v2 Protocol (PN 101-4964).

Workflow

1 Prime the IFC	2 Load cells	3 Run lysis, reverse transcription, and PCR	4 Harvest cDNA
Prepare cells and stain	Pipet and image cells, prepare pre-mixes and barcodes	Pipet mRNA Seq HT chemistry	Transfer cDNA to a 96-well plate for library preparation

How to Use the Maps

1 Select the appropriate map for your workflow step, then print on a color printer as a 1-sided page at actual size (100% scale).

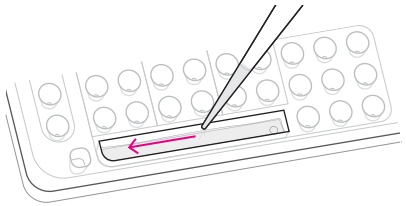


2 (Optional) Cut the image at the dotted line ( ).

3 Place the map under the HT IFC to use as a pipetting guide. 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.

Before You Pipet into the HT IFC

- 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).
- Follow the instructions for each map, and make sure to remove any remaining reagents before loading the reagents in the order shown.
- 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 ( ).



Safety

Use standard laboratory safety protocols. Read and understand the safety data sheets (SDSs) before handling chemicals. To obtain SDSs, go to fluidigm.com/sds and search for the SDS using either the product name or the part number.

For technical support visit fluidigm.com/support.

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Prime the C1 HT IFC

Reagent		Vol. (µL)	Notes
○	**1** Actuation Fluid	180	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.
●	**2** Stability Solution	20	**IMPORTANT** Stability Solution is viscous. **Do not vortex**. Make sure to use a P200 pipette.
●	**3** Valve Fluid v2	20	
●	**4** 1X Blocking Reagent	20	Make sure to dilute 10X Blocking Reagent to 1X with Cell Rinsing Reagent and keep at room temperature until use.
▭	**5** 0.01X Blocking Reagent	180	Make sure to dilute 1X Blocking Reagent to 0.01X with Cell Rinsing Reagent and keep at room temperature until use. **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.

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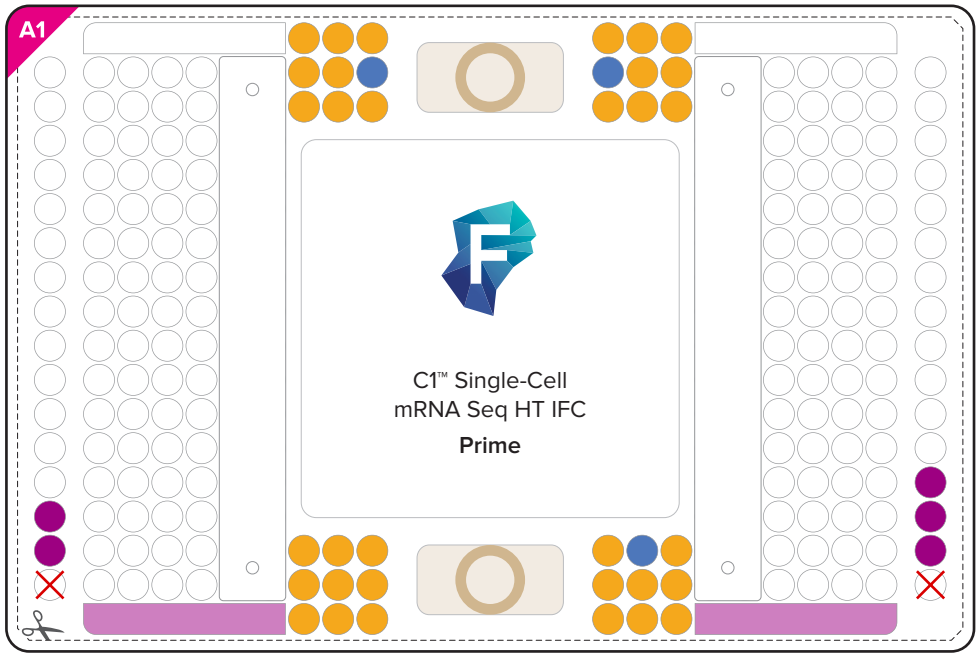

Remove Priming Reagents

IMPORTANT Before you continue to load cells, make sure to remove the following priming reagents.

Reagent	Vol. (µL)	Notes
● 1X Blocking Reagent	20	Remove any remaining reagent from each of the 4 inlets highlighted below. **IMPORTANT** After priming the IFC, you might see residual reagent in the remaining ● inlet. You do not need to remove it.

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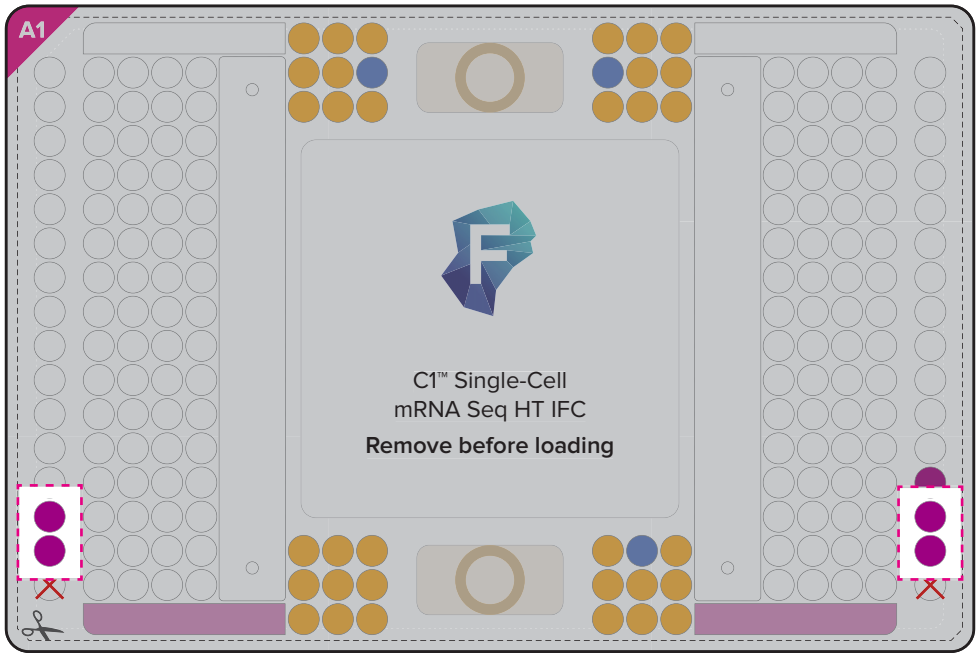

Load Cells into the C1 HT IFC

IMPORTANT Before you continue to load cells, make sure to remove the priming reagents as shown on page 3.

Reagent		Vol. (µL)	Notes
●	**1** Cell mix	10	Set a P200 pipette to 90% of the total cell mix volume, 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.** You can load the same cell mix into both cell inlets, or a different cell mix into each cell inlet.
●	**2** Stain or rinse: • **Staining cells:** LIVE/DEAD® Staining Solution • **Not staining cells:** Cell Rinsing Reagent	20	Pipet only one of the reagents into the 2 inlets labeled “stain or rinse”. • **If staining cells:** Vortex the LIVE/DEAD staining solution well, and then pipet the staining solution into the 2 stain inlets. • **If not staining cells:** Pipet the Cell Rinsing Reagent into the 2 stain inlets 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),

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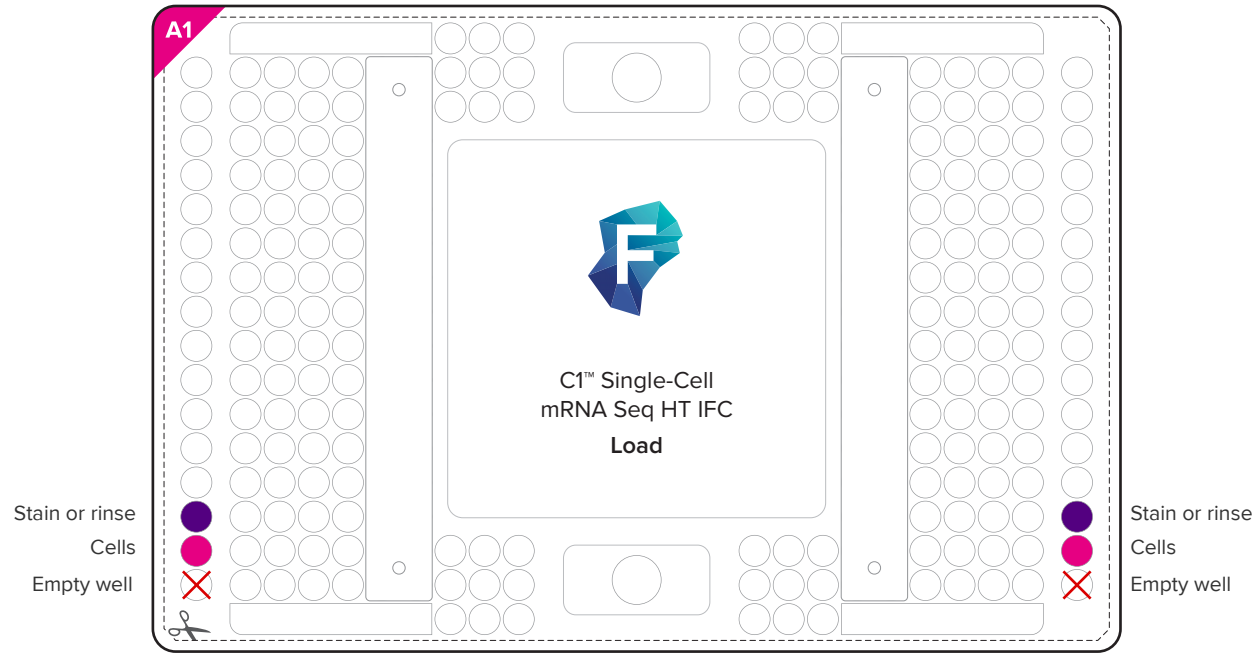

Remove Loading Reagents

IMPORTANT Before you continue to pipet run chemistry into the HT IFC, make sure to remove the following loading reagents.

Reagent		Vol. (µL)	Notes
	1 0.01X Blocking Reagent	180	Remove the reagent from the 2 wash reservoirs highlighted below. Some fluid can remain in the reservoir.
	2 Cell mix	10	Remove any remaining reagent from each of the 2 cell inlets highlighted below.
	3 Staining Solution or Cell Rinsing Reagent	20	Remove any remaining reagent from each of the 2 stain inlets highlighted below.
	4 1X Blocking Reagent	20	Remove any remaining reagent from well 4, above the cell and stain inlets at the right of the HT IFC (highlighted below).

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Pipet Lysis, Reverse Transcription, and Preamplification Chemistry into the HT IFC

IMPORTANT Before you continue to pipet run chemistry into the HT IFC, make sure to remove the loading reagents as shown on page 5. Use a fresh pipette tip for each well. Make sure to keep the HT IFC as level as possible, and evenly distribute the reagents over the bottom surface of the reservoirs.

Reagent		Vol.(µL)	Notes
	1 Preloading Reagent	180	
	2 C1 Harvest Reagent	180	
	3 Preamplification Mix (Mix C)	130	
	4 Preloading Reagent	20	
	5 RT Mix (Mix B)	20	
	6 Valve Fluid v2	20	
	7 Preloading Reagent	10	Aliquot 35 µL into each tube of an 8-tube strip, then use a multichannel pipette to pipet into each well.
1 2 3 4 5	**8** Lysis Mix A Plus Diluted Barcodes	5	Use a multichannel pipette with fresh pipette tips for each column to very carefully transfer, column by column, the diluted barcodes to each of the corresponding wells (1 2 3 4 5). **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.

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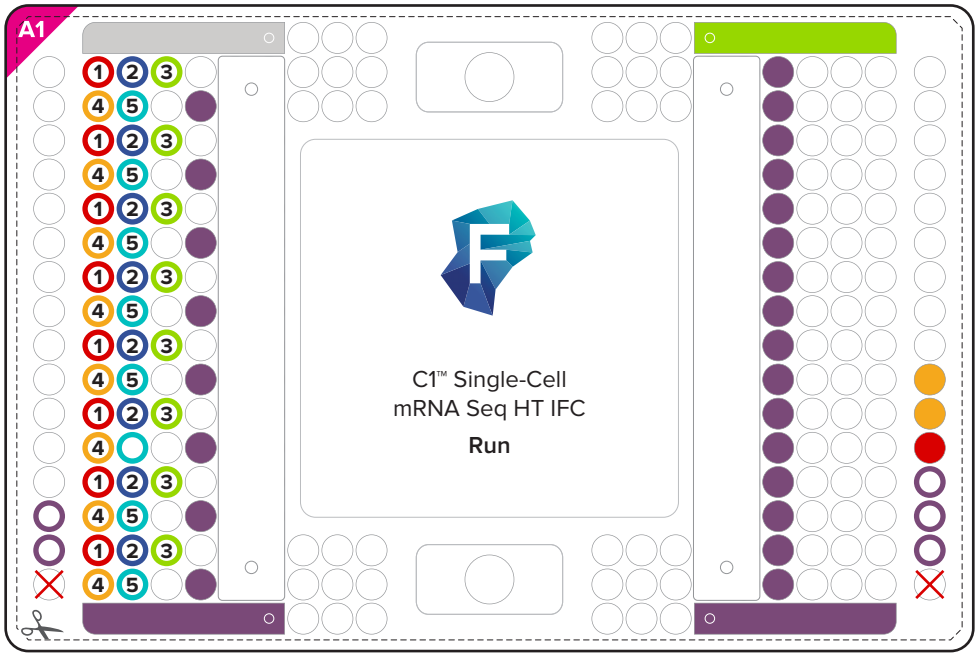

Harvest cDNA from the C1 HT IFC

Reagent	Vol. (µL)	Notes
Harvested cDNA amplicons	~ 3–6 µL	Carefully pull back the tape covering the harvesting inlets on the left and right sides of the HT IFC using the plastic removal tool. 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 96-well harvest plate containing 45 µL of 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. **STOPPING POINT** You can store the harvest plate overnight at 4 °C or immediately proceed to cleanup.

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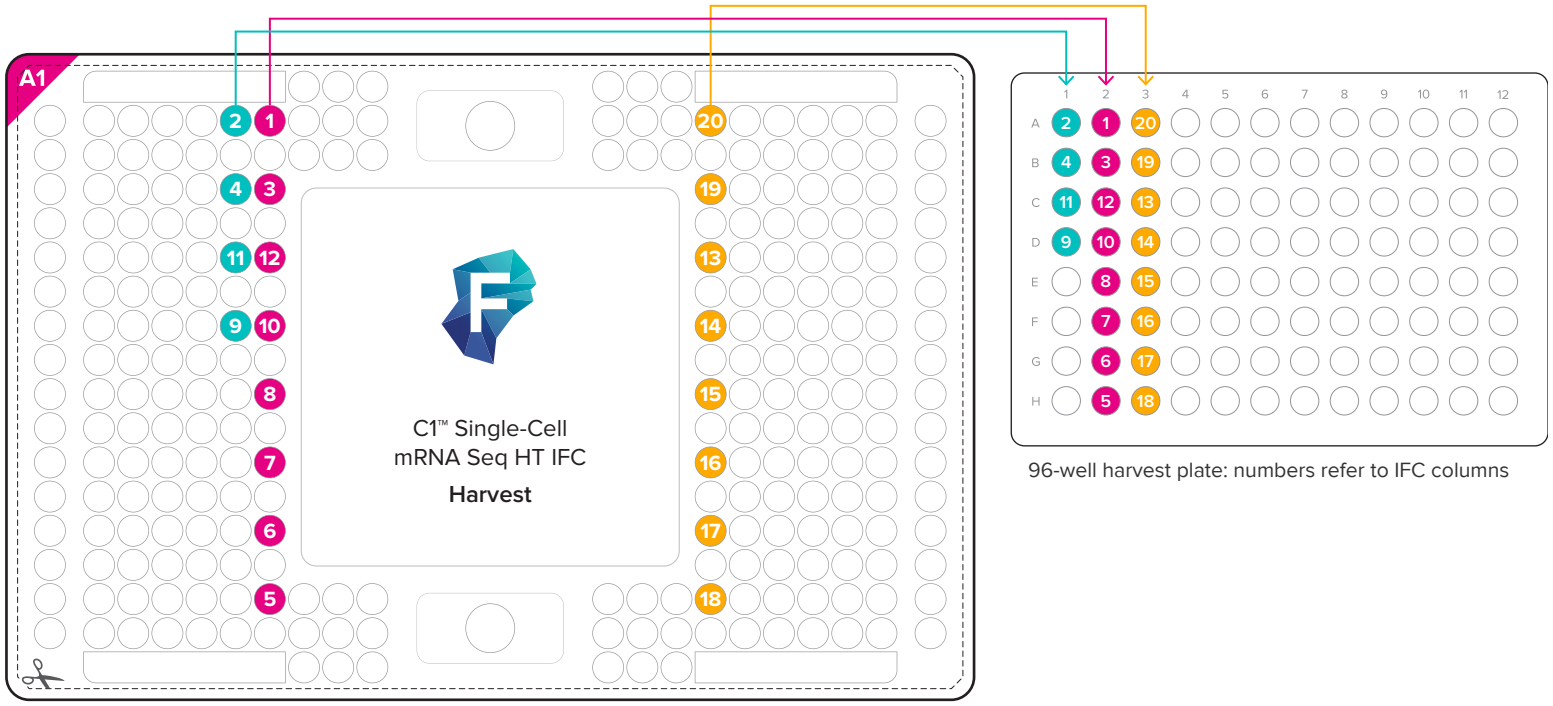

96-well harvest plate: numbers refer to IFC columns