Pipetting Maps for the C1 mRNA Seq HT Protocol

Before You Begin

Before using the maps and for detailed instructions on preparing the C1™ mRNA Seq HT chemistry, see the Using the C1 High-Throughput IFC to Generate Single-Cell cDNA Libraries for mRNA Sequencing Protocol (PN 100-9886).

Workflow

1 Prime the IFC	2 Load cells	3 Run lysis, reverse transcription, and PCR	4 Harvest cDNA
Prepare pre-mixes, cells, and stain	Pipet and image cells	Prepare 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.

Best Practices

When pipetting into the HT IFC:

- Follow the instructions for each map.
- 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 inlets. If a bubble is introduced, ensure that it floats to the top of the well.
- Make sure to evenly distribute reagents over the bottom surfaces of the accumulators and reservoirs.

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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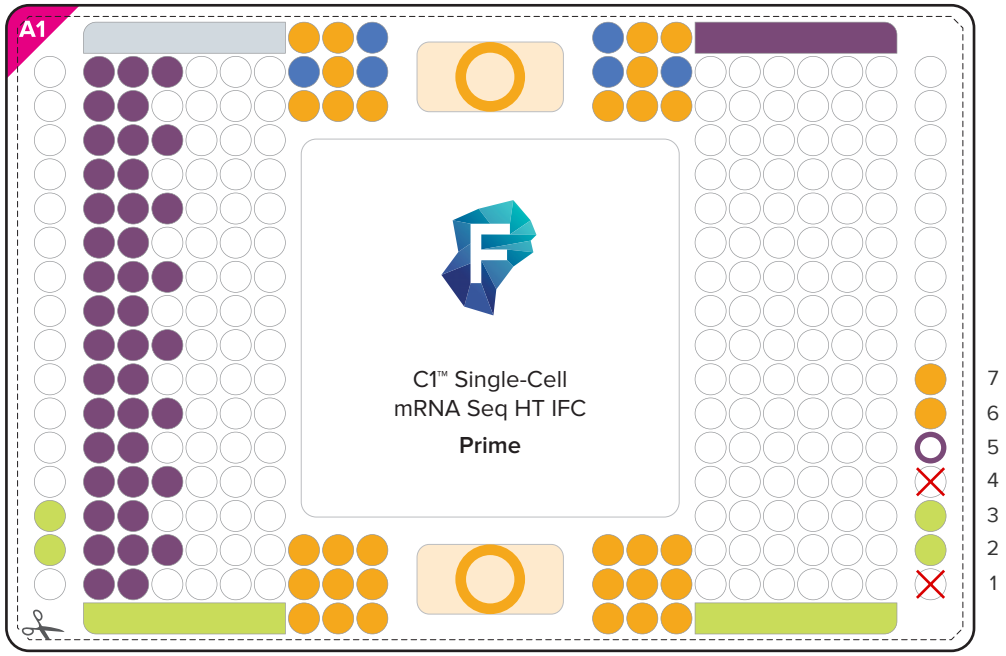
Prime the C1 HT IFC

Reagent Loading

Reagent	Vol. (µL)	Notes
Valve Fluid	250	The Valve Fluid is a high-surface-tension solution. With a P200 pipette, use 2 aliquots of 125 µL. **IMPORTANT** Make sure to keep the HT IFC as level as possible so the fluid does not move away from the center of either accumulator ( ). Gently tap the HT IFC on the bench to distribute the fluid evenly over the bottom surface of both accumulators ( ). Use just enough force to **avoid creating bubbles**.
Valve Fluid	20	
Stability Solution	20	The Stability Solution is viscous. The final volume transferred to each well may be <20 µL. **IMPORTANT** Make sure to use a P200 pipette. Pipet the solution slowly and carefully to **avoid bubbles**.

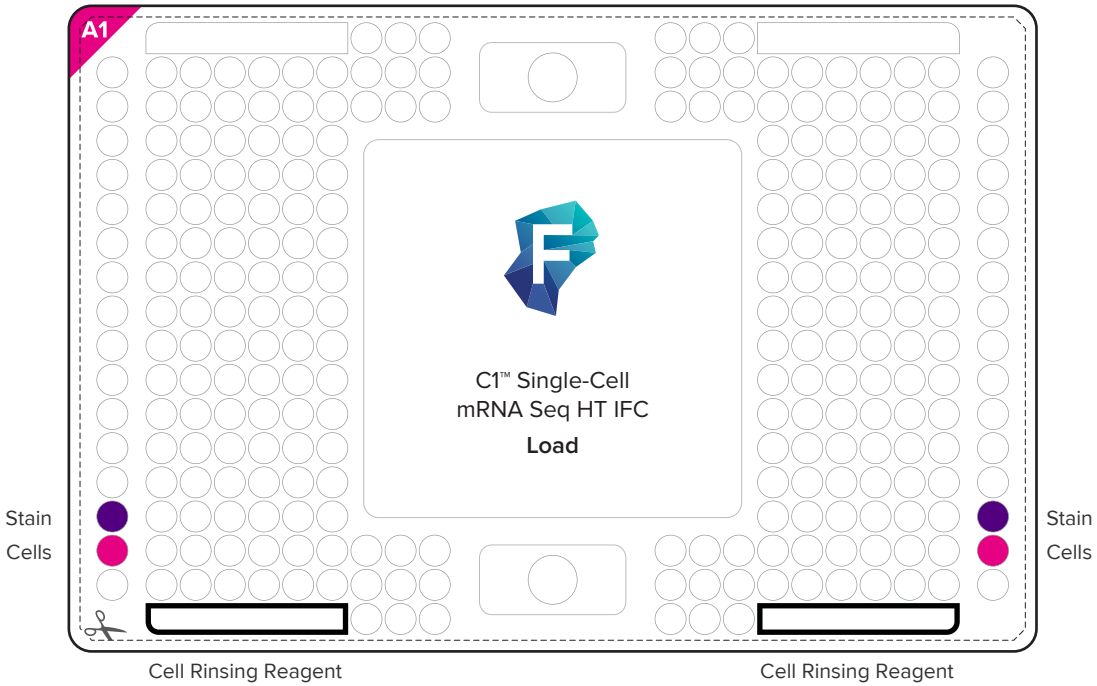
Reagent	Vol. (µL)	Notes
Preloading Reagent	5	Aliquot 30 µL into each tube of an 8-tube strip, then use a multichannel pipette to pipet into each well.
Preloading Reagent	20	
Preloading Reagent	150	
C1 Harvest Reagent	180	
1X Blocking Reagent	180	**IMPORTANT** Make sure to dilute the 10X Blocking Reagent to 1X with Cell Rinsing Reagent and keep on ice until use.
1X Blocking Reagent	20	



Load Cells into the C1 HT IFC

IMPORTANT Remove any remaining reagents in step 1 before loading the reagents in step 2.

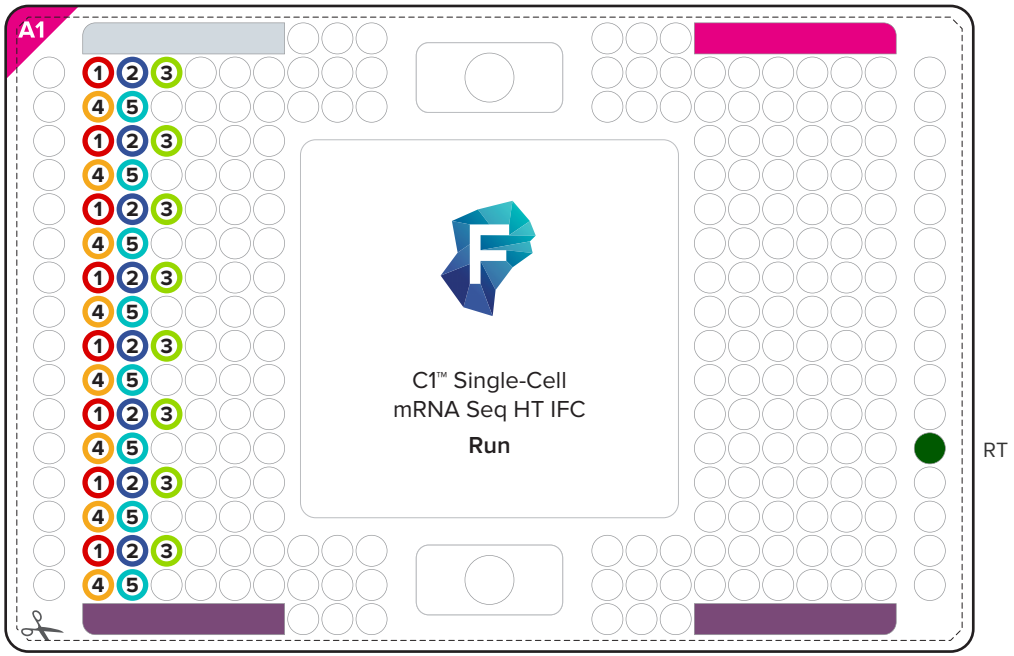
Step 1. Reagent Removal		Step 2. Reagent Loading			
Old Reagent	**Previous Map**	**New Reagent**		**Vol. (µL)**	**Notes**
1X Blocking Reagent	Prime	Cell Rinsing Reagent		180	
1X Blocking Reagent	Prime	• **Staining cells:** LIVE/DEAD® Staining Solution • **Not staining cells:** Cell Rinsing Reagent		20	Load one of these reagents.
1X Blocking Reagent	Prime	Cell mix		20	You can load the same or two different cell mixes. **IMPORTANT** Pipet the cell mix up and down 5–10 times to mix. **Do not vortex. Avoid creating bubbles.**



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

IMPORTANT Remove any remaining reagents in step 1 before loading the reagents in step 2.

Step 1. Reagent Removal		Step 2. Reagent Loading			
Old Reagent	**Previous Map**	**New Reagent**		**Vol.(µL)**	**Notes**
Preloading Reagent	Prime	Lysis Mix A Plus Diluted Barcodes		4.2	Use a multichannel pipette with fresh pipette tips for each column. **Avoid creating bubbles.** **IMPORTANT** Do not allow a pipette tip to touch another well.
Preloading Reagent	Prime	RT Mix B		20	
Preloading Reagent	Prime	Preamplification Mix I (Mix D)		130	
C1 Harvest Reagent	Prime	Cleanup Mix C		180	If you program to harvest the next day, you can store remaining Cleanup Mix C overnight on ice or at 4 °C until you are ready to harvest.
Cell Rinsing Reagent	Load	Preloading Reagent		150	



Harvest cDNA from the C1 HT IFC

Reagent Removal

Reagent	Vol. (µL)	Notes
Harvested cDNA amplicons	5	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 multichannel pipette set to 5 µL, transfer the harvested cDNA amplicons from each column of HT IFC inlets and into the corresponding wells of the 96-well harvest plate. Place the plate on ice. The volume harvested from each well may be <5 µL.