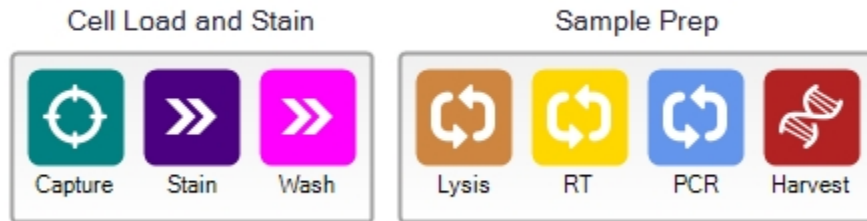




Name NEBNext mRNA Sequencing
 Revision A
 Description
 Authors Single-Cell Genomics R&D
 Institution Fluidigm Corporation
 Lab Single-Cell Genomics
 Special Instructions



Script Summary - Prime

Runtime Estimates

Barcode	Estimate
1861x (5-10 um diameter cells)	0 hours, 11 minutes
1862x (10-17 um diameter cells)	0 hours, 13 minutes
1863x (17-25 um diameter cells)	0 hours, 12 minutes
1771x (5-10 um diameter cells)	0 hours, 11 minutes
1772x (10-17 um diameter cells)	0 hours, 13 minutes
1773x (17-25 um diameter cells)	0 hours, 12 minutes

Script Summary - Cell Load and Stain

Runtime Estimates

Barcode	Estimate
1861x (5-10 um diameter cells)	0 hours, 31 minutes
1862x (10-17 um diameter cells)	1 hours, 0 minutes
1863x (17-25 um diameter cells)	0 hours, 51 minutes
1771x (5-10 um diameter cells)	0 hours, 31 minutes
1772x (10-17 um diameter cells)	1 hours, 0 minutes
1773x (17-25 um diameter cells)	0 hours, 51 minutes

Incubation Profile

Script Step	Operation	Temperature (C)	Duration (s)
Stain	Incubation	25	600

Script Summary - Sample Prep

Runtime Estimates

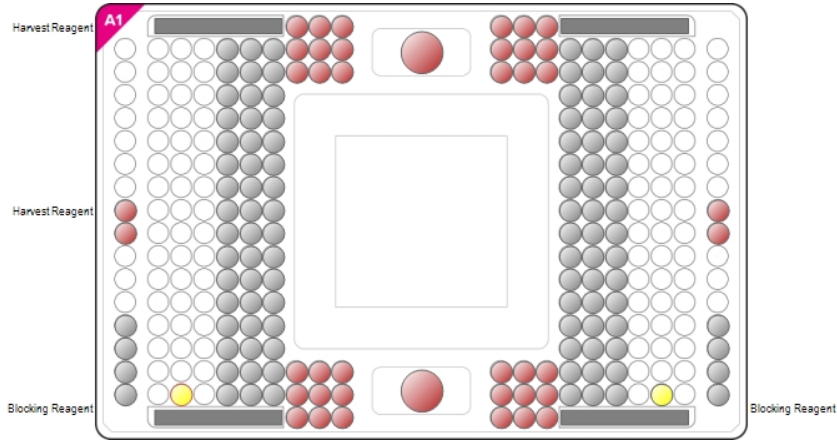
Barcode	Estimate
1861x (5-10 um diameter cells)	5 hours, 54 minutes
1862x (10-17 um diameter cells)	5 hours, 54 minutes



1863x (17-25 um diameter cells)	5 hours, 54 minutes			
1771x (5-10 um diameter cells)	5 hours, 54 minutes			
1772x (10-17 um diameter cells)	5 hours, 54 minutes			
1773x (17-25 um diameter cells)	5 hours, 54 minutes			
Incubation Profile				
Script Step	Operation		Temperature (C)	Duration (s)
Lysis	Incubation	S1	70	300
		S3	25	60
RT	Incubation	S1	42	5400
		S2	70	600
PCR	Hot Start	98C	98	45
PCR	PCR x17	Denaturation	98	10
		Annealling	62	15
		Extension	72	180
PCR	Extension	72C	72	300



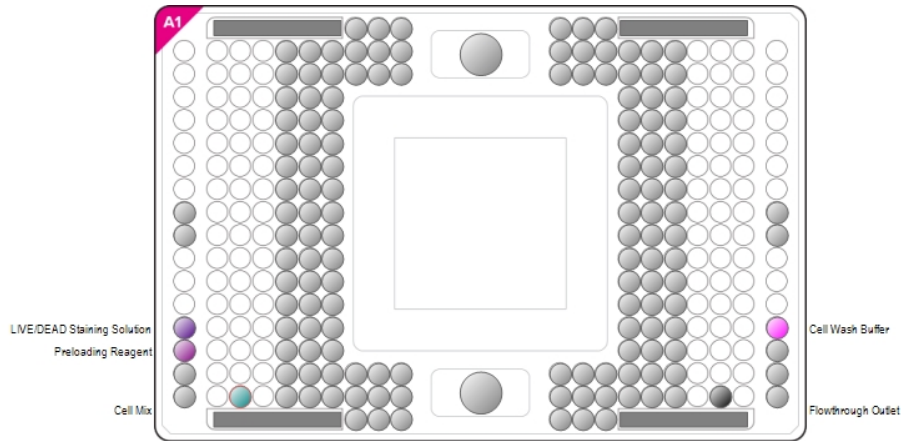
Script Reagent Details - Prime



Reagent Loading			
Name	Volume (µl)	IFC Inlet	Notes
● Harvest Reagent	200 µl	A1	
● Harvest Reagent	200 µl	A2	
● Blocking Reagent	15 µl	C1	
● Blocking Reagent	15 µl	C2	
● Harvest Reagent	20 µl	P1	
● Harvest Reagent	20 µl	P2	
Reagent Mix Recipe - Prime			
Blocking Reagent			
Reagent (Stock Concentration)	Mix Prep (µl)	Prep Conc.	Chamber Conc.
C1 Blocking RGT (1X)			
Harvest Reagent			
Reagent (Stock Concentration)	Mix Prep (µl)	Prep Conc.	Chamber Conc.
C1 Harvest RGT (1X)			



Script Reagent Details - Cell Load and Stain



Inlet Reuse			
Name	IFC Inlet	Instructions	
● Cell Mix	C1	Aspirate inlet prior to loading reagents	
● Flowthrough Outlet	C2	Aspirate inlet prior to loading reagents (1862x, 1863x only)	
Reagent Loading			
Name	Volume (µl)	IFC Inlet	Notes
● LIVE/DEAD Staining Solution	7	1	
● Preloading Reagent	24	2	
● Cell Wash Buffer	7	5	
● Cell Mix	6	C1	
Reagent Mix Recipe - Cell Load and Stain			
Preloading Reagent			
Reagent (Stock Concentration)	Mix Prep (µl)	Prep Conc.	Chamber Conc.
C1 Preloading RGT (1X)			
Cell Mix			
Reagent (Stock Concentration)	Mix Prep (µl)	Prep Conc.	Chamber Conc.
Suspension RGT (2.5X)	40	1	1
Cells 66-330 / µL	60		
100 Total Prep Volume			
LIVE/DEAD Staining Solution			
Reagent (Stock Concentration)	Mix Prep (µl)	Prep Conc.	Chamber Conc.
Cell Wash BUF (1X)	1250	0.9975	0.9975
Ethidium homodimer-1 (2 mM)	2.5	0.004	0.004
Calcein AM (4 mM)	0.625	0.002	0.002

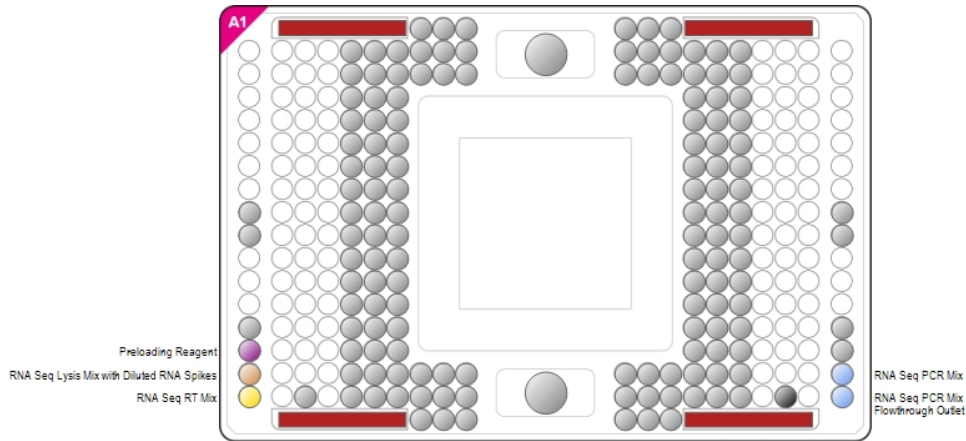


1253.125 Total Prep Volume

Cell Wash Buffer			
Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
Cell Wash BUF (1X)			



Script Reagent Details - Sample Prep



Inlet Reuse				
Name	IFC Inlet	Instructions		
● Preloading Reagent	2	Aspirate inlet prior to loading reagents		
● Flowthrough Outlet	C2	Aspirate inlet prior to loading reagents (1862x, 1863x only)		
Reagent Loading				
Name	Volume (µl)	IFC Inlet	Notes	
● Preloading Reagent	24	2		
● RNA Seq Lysis Mix with Diluted RNA Spikes	7	3		
● RNA Seq RT Mix	8	4		
● RNA Seq PCR Mix	24	7		
● RNA Seq PCR Mix	24	8		
● Harvest Reagent	180 µl each	Harvest Inlets		
Reagent Mix Recipe - Sample Prep				
Preloading Reagent				
Reagent (Stock Concentration)	Mix Prep (µl)	Prep Conc.	Chamber Conc.	
C1 Preloading RGT (1X)				
ArrayControl RNA Spikes (Secondary: 1X)				
Special Instructions:				

After the ArrayControl RNA Spikes have thawed, remove spikes 1, 4, and 7 from the box. Pipette the following in three tubes:				
Tube A - 13.5 µl THE RNA Storage Solution, RNA Spikes #7 - 1.5 µl				
Tube B - 12.5 µl THE RNA Storage Solution, RNA Spikes #4 - 1.5 µl				
Tube C - 148.5 µl THE RNA Storage Solution, RNA Spikes #1 - 1.5 µl				
Vortex briefly tube A and spin to collect contents. Pipette 1.5 µl from tube A into tube B. Discard tube A.				

Vortex briefly tube B and spin to collect contents. Pipette 1.5 µl from tube B into tube C. Discard tube B. Vortex briefly tube C and spin to collect contents.

Tube C is the concentrated RNA Standard that may be aliquoted and frozen for future use. Aliquot in tubes containing 1 µl volumes and store at -80 °C until use. One tube is necessary for each C1 chip run.

Reagent (Stock Concentration)	Mix Prep (µl)	Prep Conc.	Chamber Conc.
THE RNA Storage Solution	174		
ArrayControl RNA Spikes #1 (100 ng/µl)	1.5	0.8403	
ArrayControl RNA Spikes #4 (100 ng/µl)	1.5	0.8403	
ArrayControl RNA Spikes #7 (100 ng/µl)	1.5	0.8403	
178.5 Total Prep Volume			

RNA Seq Diluted RNA spikes (Secondary: 20X)			
Reagent (Stock Concentration)	Mix Prep (µl)	Prep Conc.	Chamber Conc.
C1 Loading RGT (20X)	99	19.8	
ArrayControl RNA Spikes (1X)	1	0.01	
100 Total Prep Volume			

RNA Seq Lysis Mix with Diluted RNA Spikes			
Reagent (Stock Concentration)	Mix Prep (µl)	Prep Conc.	Chamber Conc.
RNA Seq Diluted RNA spikes (20X)	1	1	0.667
Murine RNase Inhibitor (40 U/µl)	0.8	1.6	1.0672
NEBNext Single Cell RT Primer mix (20X)	3.5	3.5	2.3345
NEBNext Cell Lysis Buffer (10X)	3	1.5	1.0005
Nuclease Free Water	11.7		
20 Total Prep Volume			

RNA Seq RT Mix			
Reagent (Stock Concentration)	Mix Prep (µl)	Prep Conc.	Chamber Conc.
C1 Loading RGT (20X)	1.2	0.75	0.4283
NEBNext Single Cell RT buffer (4X)	14	1.75	0.9992
NEBNext template switching oligo (20X)	2.8	1.75	0.9992
NEBNext Single Cell RT enzyme mix (10X)	5.6	1.75	0.9992
Nuclease Free Water	8.4		
32 Total Prep Volume			

RNA Seq PCR Mix			
Reagent (Stock Concentration)	Mix Prep (µl)	Prep Conc.	Chamber Conc.
C1 Loading RGT (20X)	4.5	1	0.811
Nuclease Free Water	33.3		



NEBNext Single Cell cDNA PCR Master Mix (2X)	50.2	1.1156	0.9047
NEBNext Single Cell cDNA PCR Primer (10 µM)	2	0.2222	0.1802

90 Total Prep Volume

Harvest Reagent			
Reagent (Stock Concentration)	Mix Prep (µl)	Prep Conc.	Chamber Conc.
C1 Harvest RGT (1X)			

Protocol Reagent Shopping List

Reagent Name	Vendor	Part Number	Kit Part Number	Stock Concentration
Ethidium homodimer-1	Life Technologies		L-3224	2 mM
Calcein AM	Life Technologies		L-3224	4 mM
THE RNA Storage Solution	Life Technologies	AM7000		
ArrayControl RNA Spikes #1	Life Technologies		AM1780	100 ng/μl
ArrayControl RNA Spikes #4	Life Technologies		AM1780	100 ng/μl
ArrayControl RNA Spikes #7	Life Technologies		AM1780	100 ng/μl
Murine RNase Inhibitor	NEB	E6429AA	E6421L	40 U/μl
NEBNext Single Cell RT Primer mix	NEB	E6422AA	E6421L	20X
NEBNext Cell Lysis Buffer	NEB	E6428AA	E6421L	10X
Nuclease Free Water	NEB	E6433AA	E6421L	
NEBNext Single Cell RT buffer	NEB	E6423AA	E6421L	4X
NEBNext template switching oligo	NEB	E6424AA	E6421L	20X
NEBNext Single Cell RT enzyme mix	NEB	E6425AA	E6421L	10X
NEBNext Single Cell cDNA PCR Master Mix	NEB	E6426AA	E6421L	2X
NEBNext Single Cell cDNA PCR Primer	NEB	E6427AA	E6421L	10 μM

Fluidigm Reagent Kits

Reagent Name	Part Number	Stock Concentration	PN 100-8920	PN 100-6201	PN 100-5319	PN 100-7357	PN 100-8921
C1 Blocking RGT	100-5316	1X	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	
C1 Harvest RGT	100-6248	1X	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>		<input checked="" type="checkbox"/>	
C1 Preloading RGT	100-5311	1X	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	
Suspension RGT	100-5315	2.5X	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	
Cell Wash BUF	100-5314	1X	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>		
C1 Loading RGT	100-5170	20X	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>			<input checked="" type="checkbox"/>