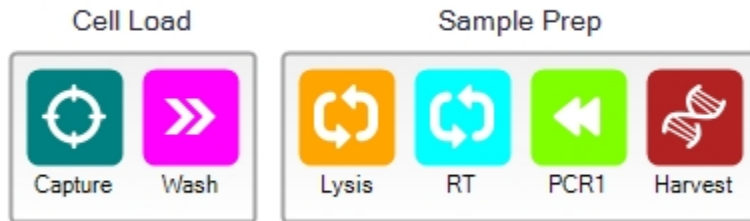




Name Total RNA-Seq  
 Revision A  
 Description Total RNA-Seq on Fluidigm C1  
 Authors Single-Cell Genomics R&D  
 Institution Fluidigm Corporation  
 Lab Single-Cell Genomics  
 Special Instructions



### 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

#### Runtime Estimates

Barcode	Estimate
1861x (5-10 um diameter cells)	0 hours, 15 minutes
1862x (10-17 um diameter cells)	0 hours, 34 minutes
1863x (17-25 um diameter cells)	0 hours, 27 minutes
1771x (5-10 um diameter cells)	0 hours, 15 minutes
1772x (10-17 um diameter cells)	0 hours, 34 minutes
1773x (17-25 um diameter cells)	0 hours, 27 minutes

### Script Summary - Sample Prep

#### Runtime Estimates

Barcode	Estimate
1861x (5-10 um diameter cells)	4 hours, 54 minutes
1862x (10-17 um diameter cells)	4 hours, 54 minutes
1863x (17-25 um diameter cells)	4 hours, 54 minutes
1771x (5-10 um diameter cells)	4 hours, 54 minutes
1772x (10-17 um diameter cells)	4 hours, 54 minutes



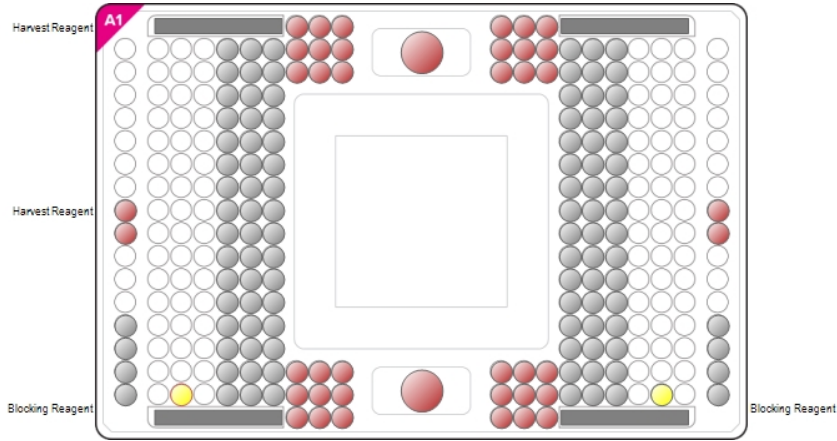
1773x (17-25 um diameter cells)      4 hours, 54 minutes

### Incubation Profile

Script Step	Operation		Temperature (C)	Duration (s)
Lysis	Lysis	RNA Fragmentation	85	360
		Cool Down	10	120
RT	RT	RT	42	5400
		RT Inactivation	70	600
		Cool Down	10	60
PCR1	Hot Start	Manual	94	60
PCR1	PCR Cycle (3 Step) x10	Denaturation	98	15
		Annealing	55	15
		Extension	68	30
PCR1	Final Extension	Extension	68	120



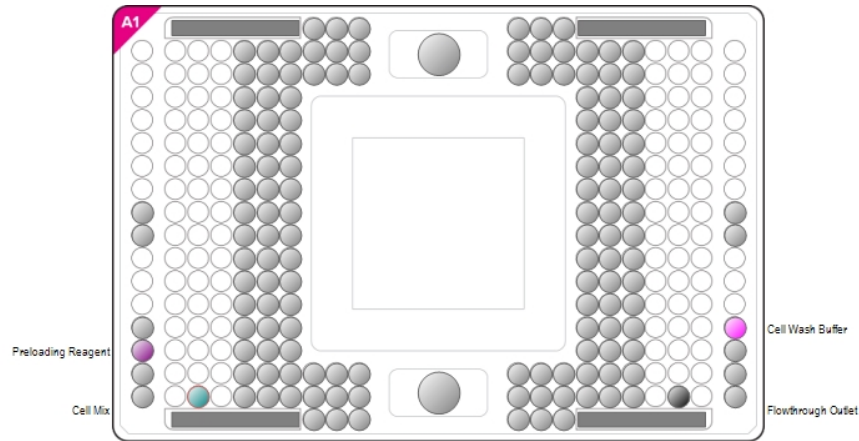
**Script Reagent Details - Prime**



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)			



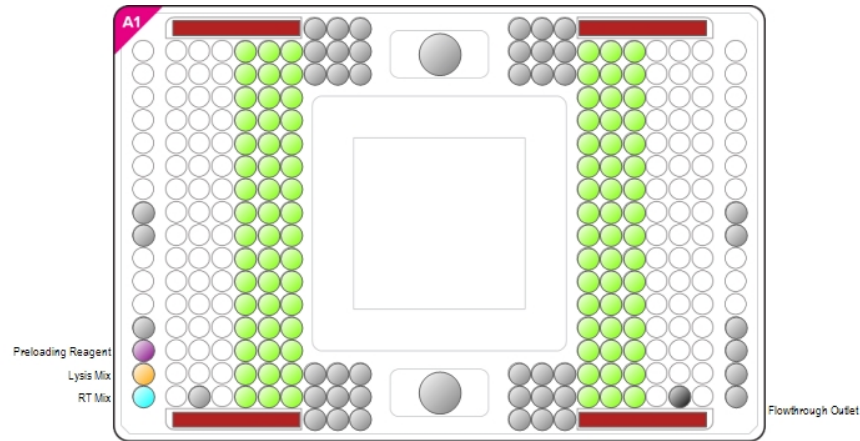
**Script Reagent Details - Cell Load**



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
● Preloading Reagent	24	2	
● Cell Wash Buffer	7	5	
● Cell Mix	↻ 6	C1	
Reagent Mix Recipe - Cell Load			
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			
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	
● Flowthrough Outlet	C2	Aspirate inlet prior to loading reagents (1862x, 1863x only)	
Reagent Loading			
Name	Volume (µl)	IFC Inlet	Notes
● Preloading Reagent	0	2	
● Lysis Mix	7	3	
● RT Mix	8	4	
● Harvest Reagent	180 µl each	Harvest Inlets	
● PCR1 Final Mix	5 each	Harvest Outlets	
Reagent Mix Recipe - Sample Prep			
Preloading Reagent			
Reagent (Stock Concentration)	Mix Prep (µl)	Prep Conc.	Chamber Conc.
C1 Preloading RGT (1X)			
Harvest Reagent			
Reagent (Stock Concentration)	Mix Prep (µl)	Prep Conc.	Chamber Conc.
C1 Harvest RGT (1X)			
10X Lysis Mix (Secondary)			
Reagent (Stock Concentration)	Mix Prep (µl)	Prep Conc.	Chamber Conc.
10X Lysis Buffer (10X)	19	9.5	
RNase Inhibitor	1		
20 Total Prep Volume			
Lysis Mix			
Reagent (Stock Concentration)	Mix Prep (µl)	Prep Conc.	Chamber Conc.

SMART scN6	1.68		
scRT Buffer	2.88		
10X Lysis Mix	1.2		
Nuclease-Free Water	3.12		
C1 Loading RGT (20X)	0.72	1.5	1.0005

9.6 Total Prep Volume

<b>RT Mix</b>			
Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
scRT Buffer	2		
SMART scTSO Mix	3.95		
RNase Inhibitor (40 U/μl)	0.5	2	1.142
SMARTScribe RT (100 U/μl)	1.75	17.5	9.9925
Nuclease-Free Water	1.3		
C1 Loading RGT (20X)	0.5	1	0.571

10 Total Prep Volume

<b>PCR1 Mix (Secondary)</b>			
Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
SeqAmp CB PCR Buffer (2X) (2X)	300	1.5625	
SeqAmp DNA Polymerase	12		
C1 Loading RGT (20X)	26.9	1.401	
Nuclease-Free Water	45.1		

384 Total Prep Volume

<b>Diluted Primer Mix (Secondary)</b>			
Special Instructions: ----- Please refer to the Technical Note (101-8496 A1) Total RNA Seq Using C1 and SMART-Seq Stranded Kit, under the section Pre-C1 Protocol for the instructions on diluting 3' and 5' primers, and the preparation of the Diluted Primer Mix plate.			
Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
Diluted 3' Primer	5		
Diluted 5' Primer	5		

10 Total Prep Volume

<b>PCR1 Final Mix</b>			
Special Instructions: ----- Aliquot 47 μL of PCR1 Mix into each well of a new 8-well PCR tube strip. Using a multi-channel pipette, distribute 3.2 μL of PCR1 Mix into each well of a new 96-well plate labeled PCR1 Final Mix. Using a multi-channel pipette, add 1.28 μL of Diluted Primer Mix from the Diluted Primer Mix plate to the corresponding wells in the PCR1 Final Mix plate.			
When it is ready to run the Total RNA Seq Sample Prep script, carefully pull back the tape covering the harvest outlets of the IFC using the plastic removal tool. Using a multi-channel pipette, transfer 4 μL of each specific			



PCR1 Final Mix from the PCR1 Final Mix plate into corresponding harvest outlets. Replace the tape to cover the harvest outlets.

Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
PCR1 Mix	4		
Diluted Primer Mix	2.08		

6.08 Total Prep Volume

**Protocol Reagent Shopping List**

Reagent Name	Vendor	Part Number	Kit Part Number	Stock Concentration
SMART scN6	Takara Bio	ST1679	634444	
scRT Buffer	Takara Bio	ST1680	634444	
10X Lysis Buffer	Takara Bio	ST1683	634444	10X
RNase Inhibitor	Takara Bio	ST1682	634444	
Nuclease-Free Water	Takara Bio	ST1287	634444	
SMART scTSO Mix	Takara Bio	ST1676	634444	
RNase Inhibitor	Takara Bio	ST1682	634444	40 U/μl
SMARTScribe RT	Takara Bio	ST1681	634444	100 U/μl
SeqAmp CB PCR Buffer (2X)	Takara Bio	ST1281	634444	2X
SeqAmp DNA Polymerase	Takara Bio	ST1280	634444	
Diluted 3' Primer	Takara Bio		634444	
Diluted 5' Primer	Takara Bio		634444	

**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"/>