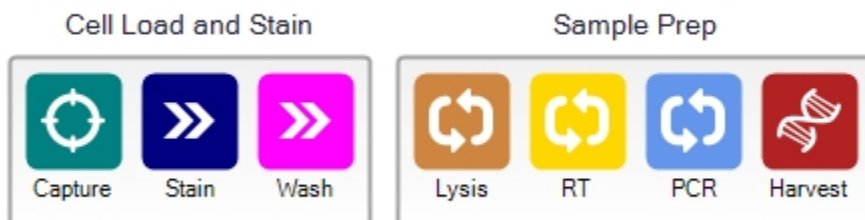


Name SMART-Seq v4  
 Revision B  
 Description The SMART-Seq v4 script, which employs a new chemistry (available in the SMART-Seq® v4 Ultra® Low Input RNA Kit for the Fluidigm® C1™ System) allows high-quality full-length cDNA synthesis from single cells.  
 Authors Sangwon Lee, Magnolia Bostick, Andrew Farmer  
 Institution Clontech Laboratories, Inc., a Takara Bio Company  
 Lab R&D  
 Special Instructions User manual is available at [www.clontech.com/SMART-Seq-for-Fluidigm](http://www.clontech.com/SMART-Seq-for-Fluidigm)



### 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

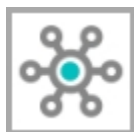
#### Incubation Profile

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

### Script Summary - Sample Prep

#### Runtime Estimates

Barcode	Estimate
---------	----------



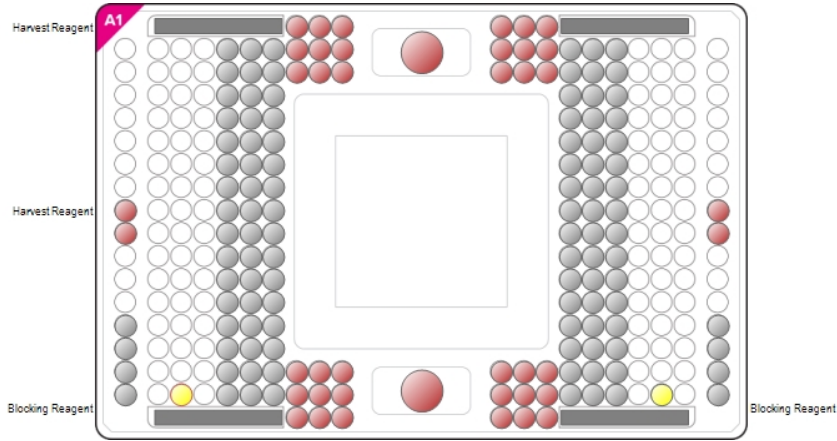
1861x (5-10 um diameter cells)	8 hours, 0 minutes
1862x (10-17 um diameter cells)	8 hours, 0 minutes
1863x (17-25 um diameter cells)	8 hours, 0 minutes
1771x (5-10 um diameter cells)	8 hours, 0 minutes
1772x (10-17 um diameter cells)	8 hours, 0 minutes
1773x (17-25 um diameter cells)	8 hours, 0 minutes

### Incubation Profile

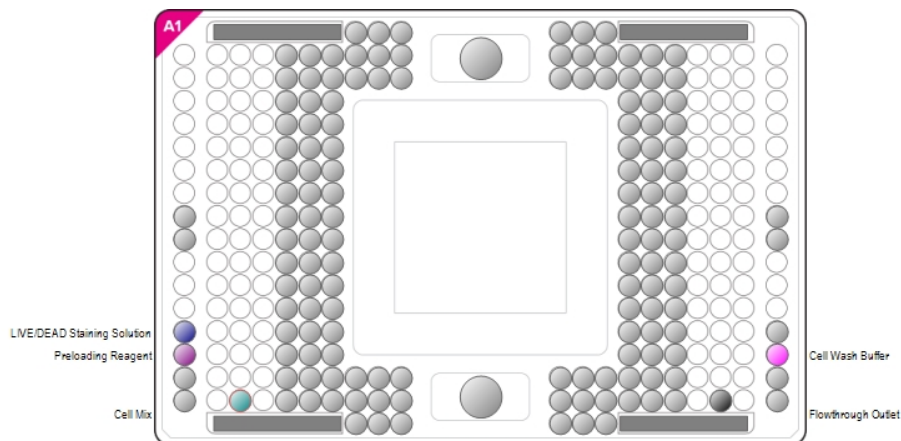
Script Step	Operation		Temperature (C)	Duration (s)
Lysis	Incubation	S1	72	180
		S2	4	600
		S3	25	60
RT	Incubation	S1	42	5400
		S2	70	600
PCR	Hot Start	98C	98	60
PCR	PCR x5	Denaturation	98	20
		Annealling	59	240
		Extension	68	360
PCR	PCR x9	Denaturation	95	20
		Annealling	65	30
		Extension	68	360
PCR	PCR x7	Denaturation	95	30
		Annealling	65	30
		Extension	68	420
PCR	Extension	72C	72	600



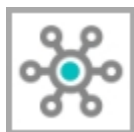
Script Reagent Details - Prime



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**


<b>Inlet Reuse</b>			
Name	IFC Inlet	Instructions	
<span style="color: teal;">●</span> Cell Mix	C1	Aspirate inlet prior to loading reagents	
<span style="color: black;">●</span> Flowthrough Outlet	C2	Aspirate inlet prior to loading reagents (1862x, 1863x only)	
<b>Reagent Loading</b>			
Name	Volume (μl)	IFC Inlet	Notes
<span style="color: blue;">●</span> LIVE/DEAD Staining Solution	7	1	
<span style="color: purple;">●</span> Preloading Reagent	24	2	
<span style="color: pink;">●</span> Cell Wash Buffer	7	6	
<span style="color: teal;">●</span> Cell Mix	6	C1	
<b>Reagent Mix Recipe - Cell Load and Stain</b>			
<b>Preloading Reagent</b>			
Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
C1 Preloading RGT (1X)			
<b>Cell Mix</b>			
Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
Suspension Reagent (2.5X)	40	1	1
Cells 66-330 / μL	60		
100 Total Prep Volume			
<b>Cell Wash Buffer</b>			
Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
Cell Wash Buffer (1X)			
<b>LIVE/DEAD Staining Solution</b>			
Special Instructions: -----			



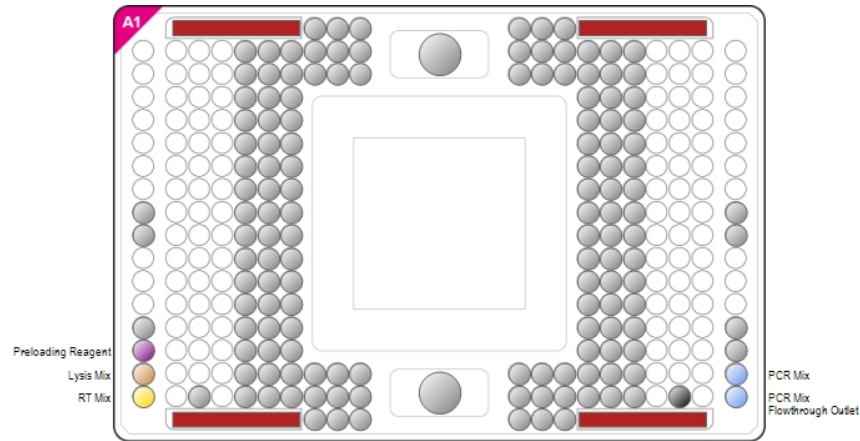
Reagents should be protected from light. Allow the reagents to warm to room temperature.  
Cell staining solution should be prepared immediately prior to load into the IFC.

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



## 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	
● Lysis Mix	7	3	
● RT Mix	8	4	
● PCR Mix	24	7	
● 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)			
10x Reaction Buffer (Secondary: 10X)			
Special Instructions:			
-----			
Lysis Buffer contains a detergent. Do not vigorously vortex lysis buffer in order to avoid bubbles.			
Reagent (Stock Concentration)	Mix Prep (µl)	Prep Conc.	Chamber Conc.
10X Lysis buffer (10X)	19	9.5	
RNase inhibitor (40 U/µl)	1	2	
20 Total Prep Volume			

Lysis Mix			
Special Instructions:			
-----			

6/23/2016 11:28:39 PM

-----  
 Thaw all reagents on ice. Gently vortex each reagent and briefly spin down before adding to a tube labeled as "Lysis Mix". Store the Lysis Mix on ice.

RNA Spikes are optional. If you are using RNA Spikes, simply replace 1  $\mu$ l of C1 loading reagent (20X) with 1  $\mu$ l of "Diluted RNA Spikes Mix", when you make a Lysis Mix. The composition of the "Diluted RNA Spikes Mix" is listed in the user manual ([www.clontech.com/SMART-Seq-for-Fluidigm](http://www.clontech.com/SMART-Seq-for-Fluidigm)).

Reagent (Stock Concentration)	Mix Prep ( $\mu$ l)	Prep Conc.	Chamber Conc.
3' SMART-Seq CDS Primer II A (12 $\mu$ M)	2.4	1.44	0.9605
Nuclease Free Water	14		
10x Reaction Buffer (10X)	2.6	1.3	0.8671
C1 Loading Reagent (20X)	1	1	0.667

20 Total Prep Volume

### RT Mix

Special Instructions:

-----  
 Thaw all the reagents (except the enzyme) on ice and warm the 5X Ultra Low First-Strand Buffer at room temperature. Vortex the 5X Ultra Low First-Strand buffer and ensure there is no precipitation in the buffer. Gently vortex each reagent to mix, spin down briefly and add to a tube labeled as "RT Mix". Add SMARTScribe Reverse Transcriptase just before loading the RT Mix on IFC. Store the RT mix on ice.

Reagent (Stock Concentration)	Mix Prep ( $\mu$ l)	Prep Conc.	Chamber Conc.
C1 Loading Reagent (20X)	1.2	0.75	0.4283
5X Ultra Low First-Strand Buffer (5X)	11.2	1.75	0.9992
SMART-Seq v4 Oligonucleotide (48 $\mu$ M)	2.8	4.2	2.3982
RNase Inhibitor (40 U/ $\mu$ l)	1.4	1.75	0.9992
Nuclease Free Water	9.8		
SMARTScribe Reverse Transcriptase (100 U/ $\mu$ l)	5.6	17.5	9.9925

32 Total Prep Volume

### PCR Mix

Special Instructions:

-----  
 Thaw all the reagents (except the enzyme) on ice. Gently vortex each reagent and briefly spin down before adding to a tube labeled as "PCR Mix". Store the PCR mix on ice.

Reagent (Stock Concentration)	Mix Prep ( $\mu$ l)	Prep Conc.	Chamber Conc.
C1 Loading Reagent (20X)	4.5	1	0.811
Nuclease Free Water	4.4		
PCR primer II A (12 $\mu$ M)	3	0.4	0.3244
SeqAmp PCR Buffer (2X)	75.2	1.6711	1.3553
SeqAmp DNA polymerase (1.25 U/ $\mu$ l)	2.9	0.0403	0.0327

90 Total Prep Volume

### Harvest Reagent

Reagent (Stock Concentration)	Mix Prep ( $\mu$ l)	Prep Conc.	Chamber Conc.
-------------------------------	---------------------	------------	---------------



C1 Harvest Reagent (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
3' SMART-Seq CDS Primer II A	Clontech	635025/635026		12 μM
Nuclease Free Water	Clontech	635025/635026		
10X Lysis buffer	Clontech	635025/635026		10X
RNase inhibitor	Clontech	635025/635026		40 U/μl
5X Ultra Low First-Strand Buffer	Clontech	635025/635026		5X
SMART-Seq v4 Oligonucleotide	Clontech	635025/635026		48 μM
SMARTScribe Reverse Transcriptase	Clontech	635025/635026		100 U/μl
PCR primer II A	Clontech	635025/635026		12 μM
SeqAmp PCR Buffer	Clontech	635025/635026		2X
SeqAmp DNA polymerase	Clontech	635025/635026		1.25 U/μl

**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 Reagent	100-5315	2.5X	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	
Cell Wash Buffer	100-5314	1X	<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 Reagent	100-5170	20X	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>			<input checked="" type="checkbox"/>
C1 Harvest Reagent	100-6248	1X	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>		<input checked="" type="checkbox"/>	