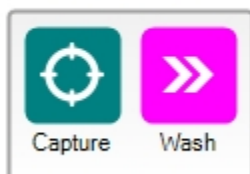
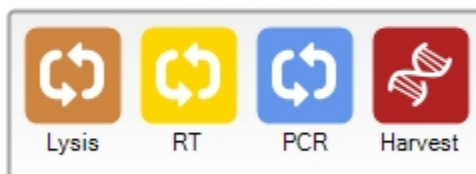


Name	SMART-Seq v4
Revision	B
Description	The SMART-Seq v4 script, which employs a new chemistry (available in the SMART-Seq® v4 Ultra® Low Input RNA Kit for the Fluidigm® C1™ System) allows high-quality full-length cDNA synthesis from single cells.
Authors	Sangwon Lee, Magnolia Bostick, Andrew Farmer
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

Cell Load



Sample Prep



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

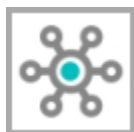
Runtime Estimates

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

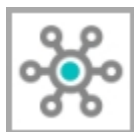
Script Summary - Sample Prep

Runtime Estimates

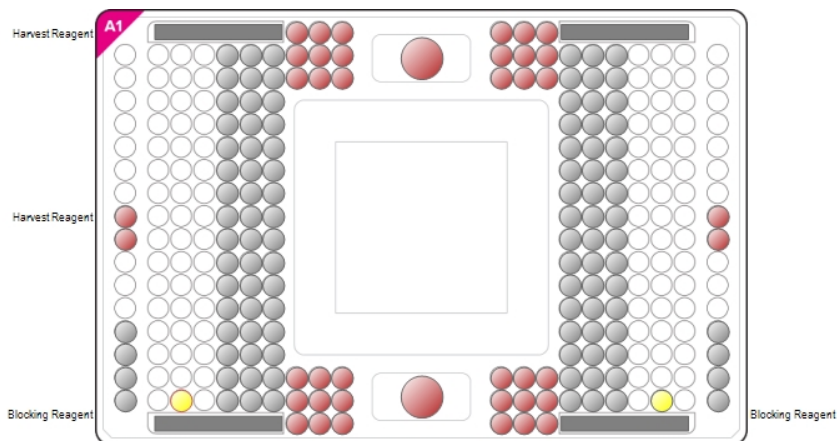
Barcode	Estimate
1861x (5-10 um diameter cells)	8 hours, 0 minutes
1862x (10-17 um diameter cells)	8 hours, 0 minutes
1863x (17-25 um diameter cells)	8 hours, 0 minutes
1771x (5-10 um diameter cells)	8 hours, 0 minutes



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	

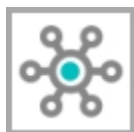
Reagent Mix Recipe - Prime

Blocking Reagent

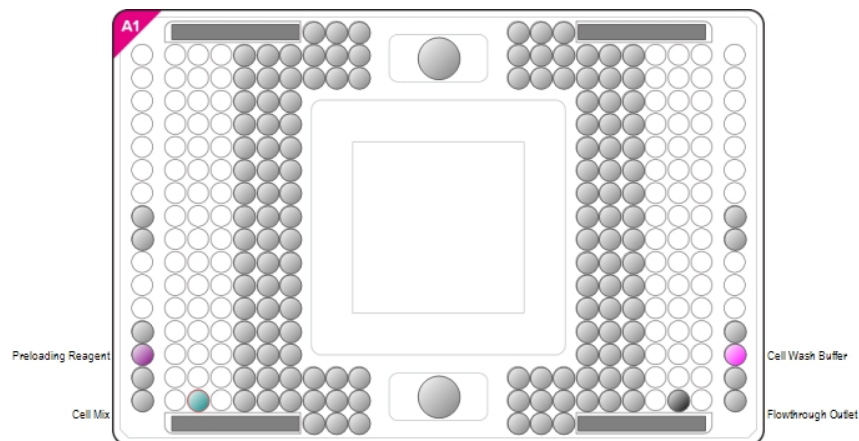
Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
C1 Blocking RGT (1X)			

Harvest Reagent

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



Script Reagent Details - Cell Load



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	6	
● 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)			

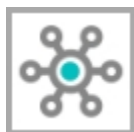
Cell Mix

Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
Suspension Reagent (2.5X)	40	1	1
Cells 66-330 / μL	60		

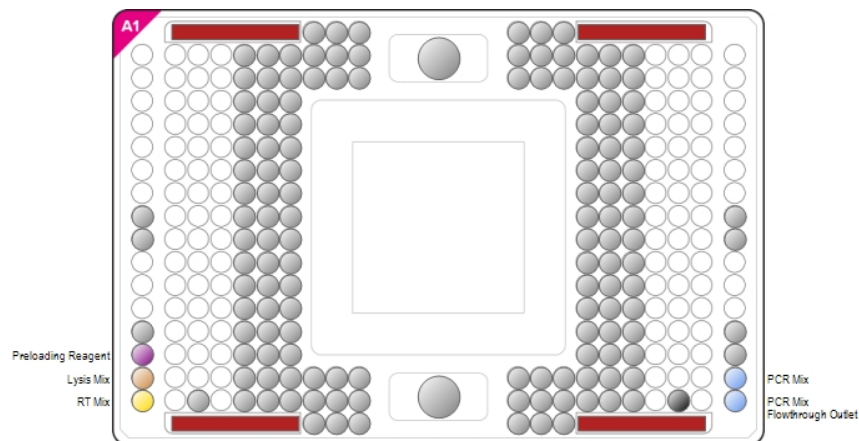
100 Total Prep Volume

Cell Wash Buffer

Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
Cell Wash Buffer (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	
● 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:

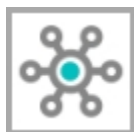
Lysis Buffer contains a detergent. Do not vigorously vortex lysis buffer in order to avoid bubbles.

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:



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 µl of C1 loading reagent (20X) with 1 µl 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).

Reagent (Stock Concentration)	Mix Prep (µl)	Prep Conc.	Chamber Conc.
3' SMART-Seq CDS Primer II A (12 µM)	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 (µl)	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 µM)	2.8	4.2	2.3982
RNase Inhibitor (40 U/µl)	1.4	1.75	0.9992
Nuclease Free Water	9.8		
SMARTScribe Reverse Transcriptase (100 U/µl)	5.6	17.5	9.9925

32 Total Prep Volume

PCR Mix

Special Instructions:

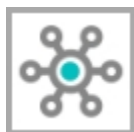
Thaw all the reagents (except the enzyme) on ice. Gently vortex each reagent and briefly spin down before adding to a tube labeled as "PCR Mix". Store the PCR mix on ice.

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

90 Total Prep Volume

Harvest Reagent

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

**Protocol Reagent Shopping List**

Reagent Name	Vendor	Part Number	Kit Part Number	Stock Concentration
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 uM
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 100-7357	PN 100-5319	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"/>	
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"/>		