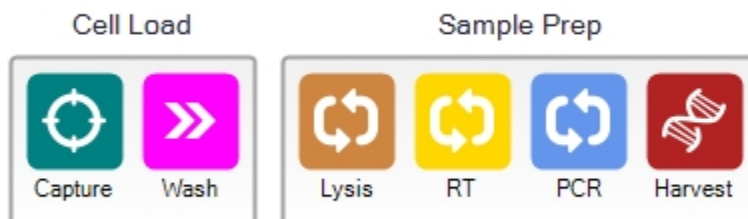


Name Smart-seq2  
 Revision C  
 Description  
 Authors Karene Argoud & Esther Mellado  
 Institution Wellcome Trust Centre for Human Genetics  
 Lab Oxford Genomics Centre  
 Special Instructions



### 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

### 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

### Script Summary - Sample Prep

#### Runtime Estimates

Barcode	Estimate
1861x (5-10 um diameter cells)	8 hours, 6 minutes
1862x (10-17 um diameter cells)	8 hours, 6 minutes
1863x (17-25 um diameter cells)	8 hours, 6 minutes

#### Incubation Profile

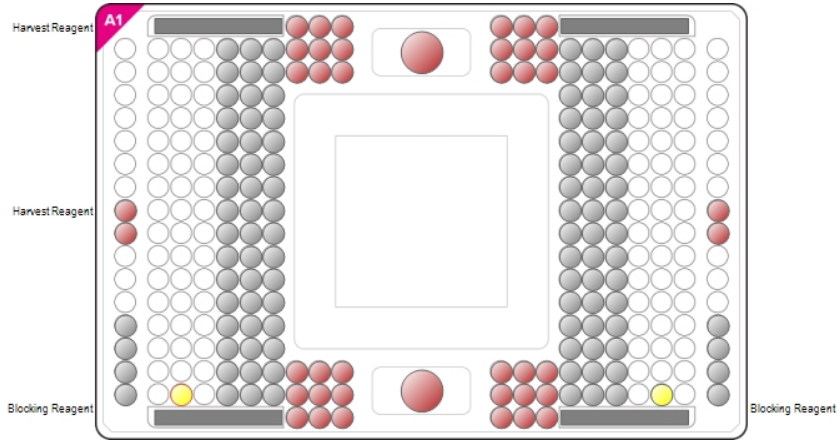
Script Step	Operation		Temperature (C)	Duration (s)
Lysis	Incubation	S1	72	180
		S2	4	600
		S3	25	60
RT	Incubation	RT and template-switching	42	5400



RT	PCR Cycle x10	Unfolding RNA secondary structures	50	120
		Completion RT and template-switching	42	120
RT	Incubation	Enzyme inactivation	70	900
RT	Hold	Hold at 6C	6	300
PCR	Hot Start	98C Denaturation	98	180
PCR	PCR x18	Denaturation	98	20
		Annealling	67	15
		Extension	72	360
PCR	Extension	72C Final extension	72	300
PCR	Hold	Hold at 10C	10	600



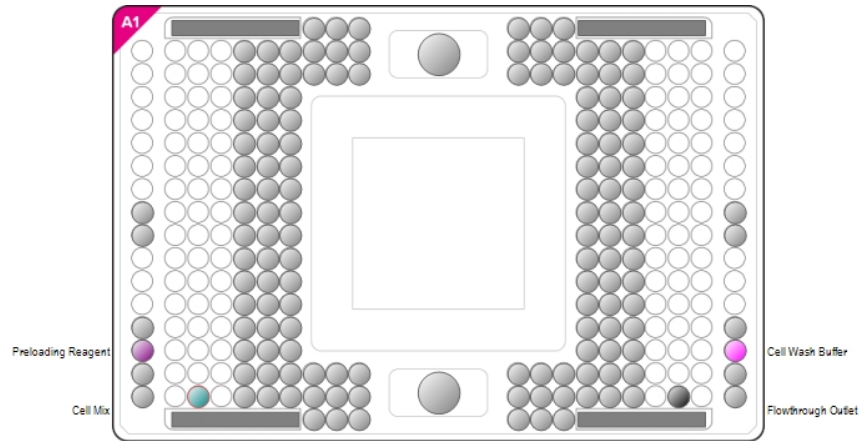
Script Reagent Details - Prime



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.
Not Defined			
Harvest Reagent			
Reagent (Stock Concentration)		Mix Prep (µl)	Prep Conc. Chamber Conc.
Not Defined			



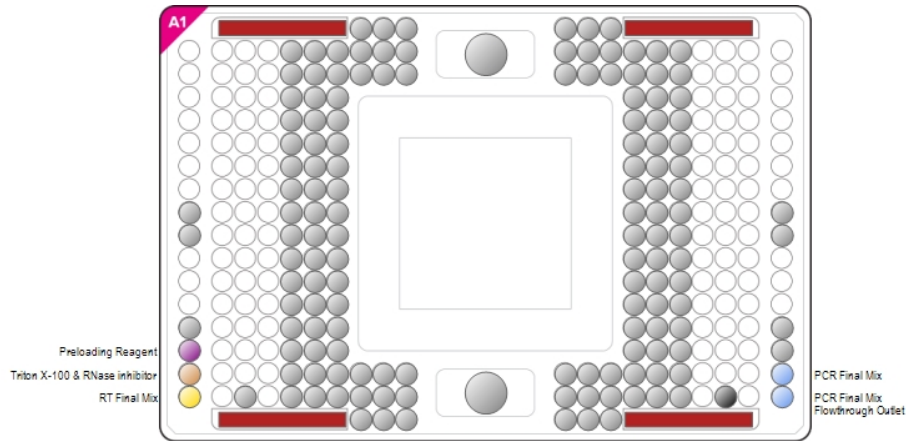
## Script Reagent Details - Cell Load



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	20	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.
Not Defined			
Cell Mix			
Special Instructions:			
-----			
The ratio of cells to cell suspension reagent should be optimized to achieve neutral buoyancy of the cells.			
Reagent (Stock Concentration)	Mix Prep (µl)	Prep Conc.	Chamber Conc.
Cells 66 - 330 / µL	60		
Suspension RGT (2.5X)	40	1	1
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	
● Triton X-100 & RNase inhibitor	7	3	
● RT Final Mix	8	4	
● PCR Final Mix	24	7	
● PCR Final 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.
Not Defined			
Triton X-100 & RNase inhibitor (Secondary)			
Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
RNase Inhibitor (40 U/μl)	2	3.6364	
0.4% Triton X-100 (vol/vol) in water	20		
22 Total Prep Volume			
Triton X-100 & RNase inhibitor			
Special Instructions:			
-----			
Oligo dT30VN sequence: 5'-AAGCAGTGGTATCAACGCAGAGTAC[dT]30VN-3'			
Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.

Oligo-dT30VN (10 µM)	7	2.5926	1.7293
dNTPs (10 mM each) (10 mM)	7	2.5926	1.7293
Triton X-100 & RNase inhibitor	12		
C1 Loading RGT (20X)	1	0.7407	0.4941

27 Total Prep Volume

**RT Final Mix**

Special Instructions:

 -----  
 TSO sequence 5'-AAGCAGTGGTATCAACGCAGAGTACATrGrG+G-3'

Reagent (Stock Concentration)	Mix Prep (µl)	Prep Conc.	Chamber Conc.
SuperScript II first strand buffer (5X)	11.2	1.7178	0.9809
SuperScript II RT (200 U/µl)	5.6	34.3558	19.6172
Rnase inhibitor (40 U/µl)	1.4	1.7178	0.9809
DTT (100 mM)	1.4	4.2945	2.4521
Betaine (1M)	5.7	0.1748	0.0998
MgCl <sub>2</sub> (1M)	0.5	0.0153	0.0088
Template Switch Oligo (TSO) (100 µM)	5.6	17.1779	9.8086
C1 Loading RGT (20X)	1.2	0.7362	0.4204

32.6 Total Prep Volume

**PCR Final Mix**

Special Instructions:

 -----  
 IS PCR primer sequence 5'-AAGCAGTGGTATCAACGCAGAGT-3'

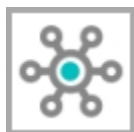
Remember the 50X dNTPs are in the KAPA HiFi Hot start ready mix. They do not need to be added separately.

Reagent (Stock Concentration)	Mix Prep (µl)	Prep Conc.	Chamber Conc.
Water	29		
KAPA HiFi Hot Start Ready Mix (2X)	50	1.1429	0.9269
IS primer (10 µM)	4	0.4571	0.3707
C1 Loading RGT (20X)	4.5	1.0286	0.8342

87.5 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
Oligo-dT30VN	Oligo Vendor			10 µM
dNTPs (10 mM each)	Invitrogen			10 mM
RNase Inhibitor	Clontech	2313A		40 U/µl
SuperScript II first strand buffer	Invitrogen	18064014		5X
SuperScript II RT	Invitrogen	18064014		200 U/µl
DTT	Invitrogen	18064014		100 mM
Betaine	Sigma	61962		1M
MgCl2	Sigma			1M
Template Switch Oligo (TSO)	Exiquon			100 µM
KAPA HiFi Hot Start Ready Mix	KAPA biosystems	KK2601		2X
IS primer	Oligo Vantor			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
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"/>
C1 Harvest RGT	100-6248	1X	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>		<input checked="" type="checkbox"/>	