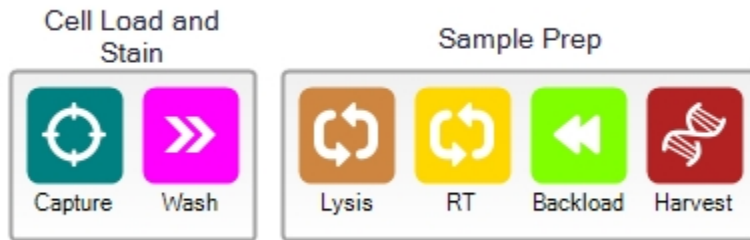




Name SMARTer single cell total RNA sequencing  
 Revision A  
 Description SMARTer single cell total RNA is a method that captures polyA+ as well as polyA-transcripts using random primers. Ribosomal cDNA is removed using specific probes.  
 Authors Verboom K. et al.  
 Institution Ghent University  
 Lab Center for Medical Genetics Ghent  
 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 and Stain

#### 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)	4 hours, 55 minutes
1862x (10-17 um diameter cells)	4 hours, 55 minutes
1863x (17-25 um diameter cells)	4 hours, 55 minutes

#### Incubation Profile

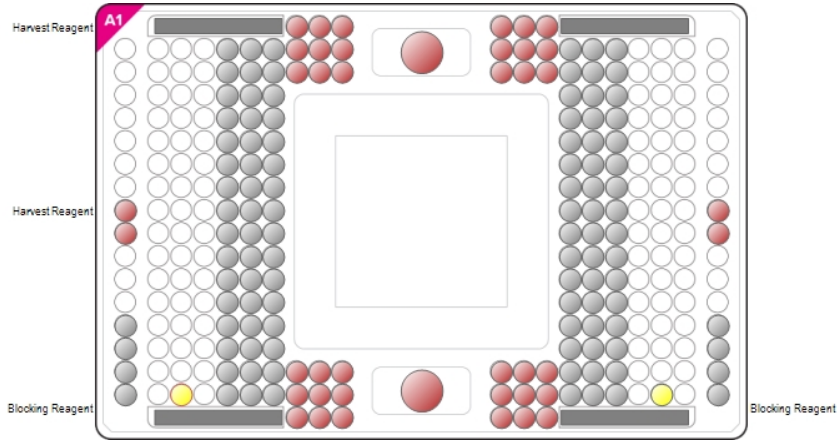
Script Step	Operation		Temperature (C)	Duration (s)
Lysis	Incubation	S1	85	360
		S2	10	120
RT	Incubation	S1	42	5400
		S2	70	600
Backload	New	Manual	94	60



Backload	PCR Cycle (3 Step) x10	Denaturation	98	30
		Annealling	55	15
		Extension	68	30
Backload	Manual	Manual	68	120



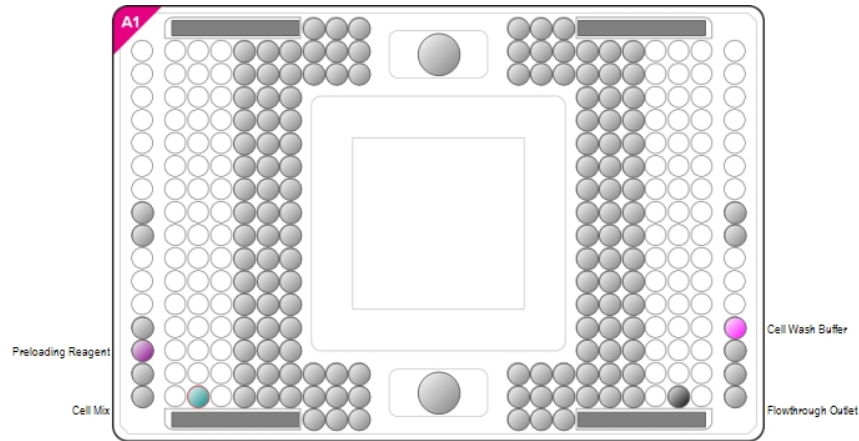
**Script Reagent Details - Prime**



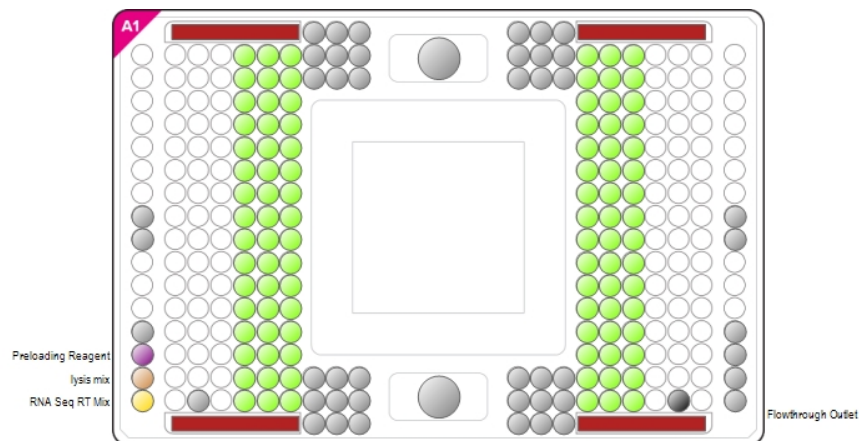
Reagent Loading			
Name	Volume (µl)	IFC Inlet	Notes
● Harvest Reagent	200 µl	A1	
● Harvest Reagent	200 µl	A2	
● Blocking Reagent	15 µl	C1	
● Blocking Reagent	15 µl	C2	
● Harvest Reagent	20 µl	P1	
● Harvest Reagent	20 µl	P2	
Reagent Mix Recipe - Prime			
Blocking Reagent			
Reagent (Stock Concentration)	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 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
● 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 300 cells / 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**


<b>Inlet Reuse</b>			
Name	IFC Inlet	Instructions	
● Flowthrough Outlet	C2	Aspirate inlet prior to loading reagents (1862x, 1863x only)	
<b>Reagent Loading</b>			
Name	Volume (μl)	IFC Inlet	Notes
● Preloading Reagent	0	2	
● lysis mix	7	3	
● RNA Seq RT Mix	8	4	
● Harvest Reagent	180 μl each	Harvest Inlets	
● PCR mix	5 each	Harvest Outlets	
<b>Reagent Mix Recipe - Sample Prep</b>			
<b>Preloading Reagent</b>			
Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
C1 Preloading RGT (1X)			
<b>10x reaction buffer (Secondary: 10X)</b>			
Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
10 X Lysis buffer (10X)	19	9.5	
RNase inhibitor (40 U/μl)	1	2	
20 Total Prep Volume			
<b>lysis mix</b>			
Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
SMART Pico Oligos Mix v2	2.3		
5XFirst-Strand Buffer (5X)	6	1.5	1.0005
RNase free H2O	6.7		

C1 Loading RGT (20X)	1	1	0.667
10x reaction buffer (10X)	3	1.5	1.0005
spikes 1/1000	1		

20 Total Prep Volume

<b>RNA Seq RT Mix</b>			
Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
C1 Loading RGT (20X)	1	1	0.571
5X First-Strand Buffer (5X)	4	1	0.571
RNase Inhibitor (40 U/μl)	0.9	1.8	1.0278
SMARTScribe Reverse Transcriptase (100 U/μl)	3.5	17.5	9.9925
SMART TSO mix v2	7.9		
RNase free H2O	2.7		

20 Total Prep Volume

<b>3' PCR primer mix (Secondary)</b>			
Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
3' PCR primer (12 μM) (12 μM)	1	2.4	
RNase free H2O	4		

5 Total Prep Volume

<b>5' PCR primer mix (Secondary)</b>			
Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
Rev primer (12 μM) (12 μM)	1	2.4	
RNase free H2O	4		

5 Total Prep Volume

<b>1,5x PCR mix (Secondary: 1.5X)</b>			
Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
2X SeqAmp CB PCR buffer (2X)	1050	1.5	
SeqAmp DNA polymerase	42		
RNase free H2O	308		

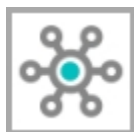
1400 Total Prep Volume

<b>PCR mix</b>			
Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
C1 Loading RGT (20X)	1	1.105	0.9901
3' PCR primer mix	2		
5' PCR primer mix	2		
1,5x PCR mix (1.5X)	13.1	1.0856	0.9727

18.1 Total Prep Volume



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

**Protocol Reagent Shopping List**

Reagent Name	Vendor	Part Number	Kit Part Number	Stock Concentration
SMART Pico Oligos Mix v2	Takara		634413	
5XFirst-Strand Buffer	Takara		634413	5X
10 X Lysis buffer	Takara	635013		10X
RNase inhibitor	Takara		634413	40 U/μl
spikes 1/1000	Thermo Fisher Scientific		4456740	
5X First-Strand Buffer	Takara		634413	5X
SMARTScribe Reverse Transcriptase	Takara		634413	100 U/μl
SMART TSO mix v2	Takara		634413	
3' PCR primer (12 μM)	Takara		634413	12 μM
Rev primer (12 μM)	Takara		634413	12 μM
2X SeqAmp CB PCR buffer	Takara		634413	2X
SeqAmp DNA polymerase	Takara		634413	

**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"/>