Name	sc-GEM
Revision	A
Description	sc-GEM
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Lab	Microfluidics Systems Biology Lab
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, 12 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, 30 minutes
1863x (17-25 um diameter cells)	0 hours, 27 minutes

Script Summary - Sample Prep

Runtime Estimates

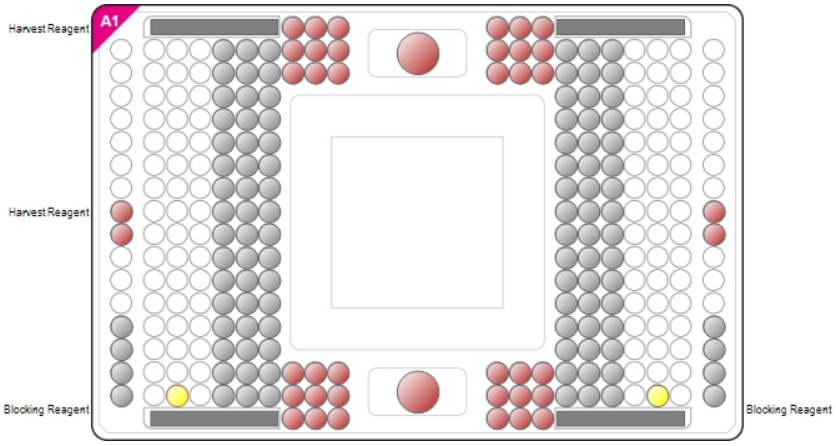
Barcode	Estimate
1861x (5-10 um diameter cells)	11 hours, 8 minutes
1862x (10-17 um diameter cells)	11 hours, 8 minutes
1863x (17-25 um diameter cells)	11 hours, 8 minutes

Incubation Profile

Script Step	Operation		Temperature (C)	Duration (s)
Lysis	Lysis	Manual	25	300
		lysis	70	600
		New Slice	25	30
RT	RT	S1	25	600
		S2	42	3600
		New Slice	70	900

		New Slice	25	30
Protease	Protease	Protease	25	10
		New Slice	50	5400
		New Slice	70	1800
		New Slice	25	30
RD	RD	S1	25	10
		New Slice	37	7200
		New Slice	80	1200
		New Slice	25	30
PreAmp	Pre-Amp	Manual	95	600
PreAmp	PCR Cycle (3 Step) x22	Denaturation	95	15
		Annealling	60	240

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

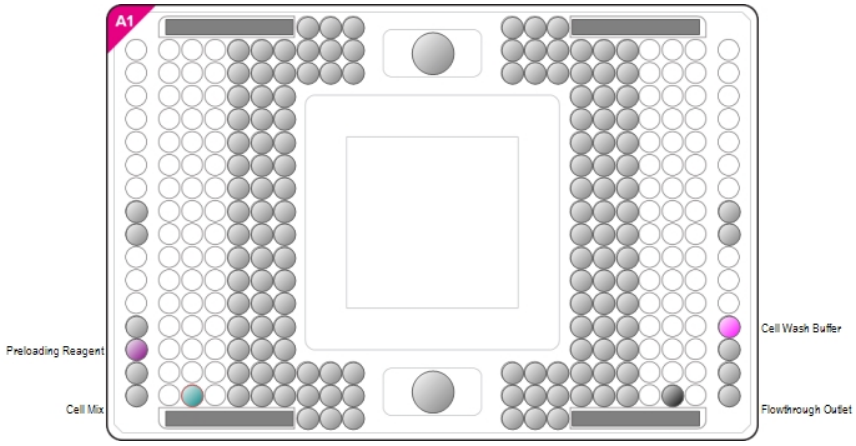
Blocking Reagent

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	5	
Cell Mix	5	C1	

Reagent Mix Recipe - Cell Load

Preloading Reagent

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

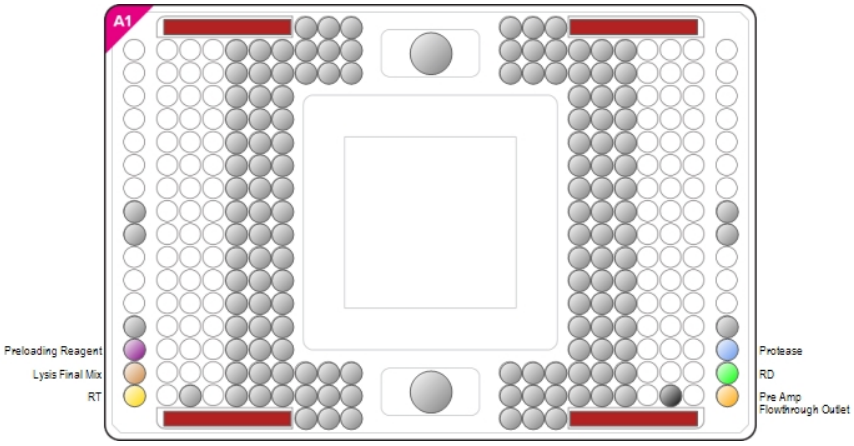
Cell Mix

Reagent (Stock Concentration)	Mix Prep (µl)	Prep Conc.	Chamber Conc.
Cells 66-330 / µL			

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
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	20	2	
Lysis Final Mix	7	3	
RT	7	4	
Protease	7	6	
RD	24	7	
Pre Amp	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			

Lysis Final Mix

Reagent (Stock Concentration)	Mix Prep (µl)	Prep Conc.	Chamber Conc.
Single-Cell Lysis Solution (2)	12.75	1.4167	0.9449
C1 Lysis Plus RGT	4.35		
PCR Water	0.9		
	18 Total Prep Volume		

RT

Reagent (Stock Concentration)	Mix Prep (µl)	Prep Conc.	Chamber Conc.
Stop solution (15)	2.91	2.425	0.97
Single-Cell VILO RT Mix (5)	8.76	2.4333	0.9733
C1 Loading RGT (20X)	0.9	1	0.4
Single-Cell PreAmp Mix (5)	5.43	1.5083	0.6033
	18 Total Prep Volume		

Protease			
Reagent (Stock Concentration)	**Mix Prep (µl)**	**Prep Conc.**	**Chamber Conc.**
C1 Loading RGT (20X)	2	1	0.286
PCR Water	18		
QIAGEN Protease (5 mg/ml)	20	2.5	0.715
	40 Total Prep Volume		

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

RD			
Reagent (Stock Concentration)	**Mix Prep (µl)**	**Prep Conc.**	**Chamber Conc.**
NEB Cutsmart Buffer (10X)	4	1.1111	0.9011
PCR Water	28		
HpaII (50 Units/ul)	4	5.5556	4.5056
	36 Total Prep Volume		

Pre Amp			
Special Instructions: --- To create the preamplification primer mix, all preamplification primers to be used should be combined and diluted in nuclease free water to a final concentration of 250 nM each primer.			
Reagent (Stock Concentration)	**Mix Prep (µl)**	**Prep Conc.**	**Chamber Conc.**
C1 PreAmp Dilution RGT	16		
Single-Cell PreAmp Mix (5)	8	1	0.448
Preamplification Primer mix (250 nM each primer) (250 nM)	16	100	44.8
	40 Total Prep Volume		

Protocol Reagent Shopping List

Reagent Name	Vendor	Part Number	Kit Part Number	Stock Concentration
Single-Cell Lysis Solution	Life Technologies		4458237	2
Stop solution	Life Technologies		4458237	15
Single-Cell VILO RT Mix	Life Technologies		4458237	5
Single-Cell PreAmp Mix	Life Technologies		4458237	5
QIAGEN Protease	QIAGEN	19155		5 mg/ml
NEB Cutsmart Buffer	New England BioLabs	B7204S		10X
HpaII	New England BioLabs	R0171M		50 Units/ul

Fluidigm Reagent Kits

Reagent Name	Part Number	Stock Concentration	PN 100-5319	PN 100-8920	PN 100-6201	PN 100-8921	PN 100-7357
Cell Wash BUF	100-5314	1X	☑	☑	☑		
C1 Lysis Plus RGT	100-5313		☑				
PCR Water	100-5941						☑
C1 Loading RGT	100-5170	20X		☑	☑	☑	
C1 Harvest RGT	100-5310	1X	☑				
C1 PreAmp Dilution RGT	100-5318		☑				