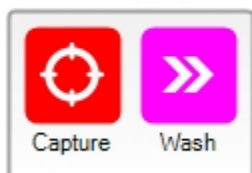


Name C1 T-ATAC sequencing
 Revision A
 Description Transcribed Indexed ATAC-seq
 Authors Anuman Satpathy, Howard Chang et al
 Institution Stanford School of Medicine, Stanford, CA
 Lab Howard Y Chang
 Special Instructions For use with C1 96 Open App integrated fluidic circuits (IFCs)

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

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)	5 hours, 17 minutes
1862x (10-17 um diameter cells)	5 hours, 17 minutes
1863x (17-25 um diameter cells)	5 hours, 17 minutes

Incubation Profile

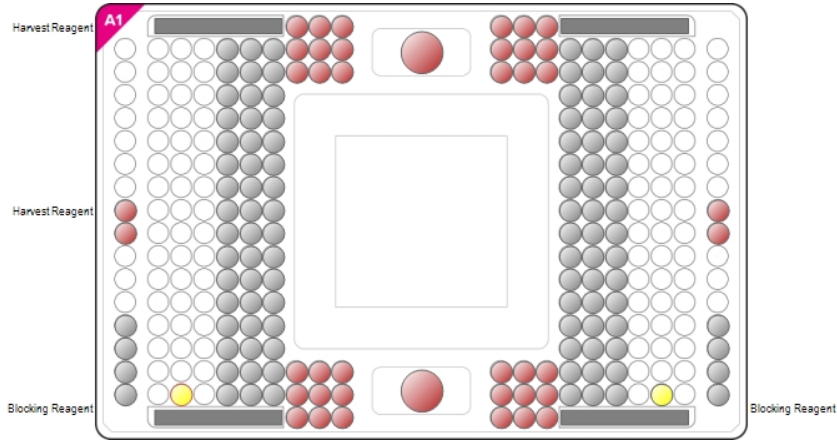
Script Step	Operation		Temperature (C)	Duration (s)
Lysis	Lysis / ATAC	Manual	37	1800
Transposase release	Incubation		50	1800
TCR_RT	TCR RT	Manual	50	1800
TCR_ATA_C_PCR	Gap filling	72C	72	300
TCR_ATA_C_PCR	PCR x8	Denaturation	94	30
		Annealing	62	60



		Extension	72	60
TCR_ATAC_PCR	Manual	Manual	72	600



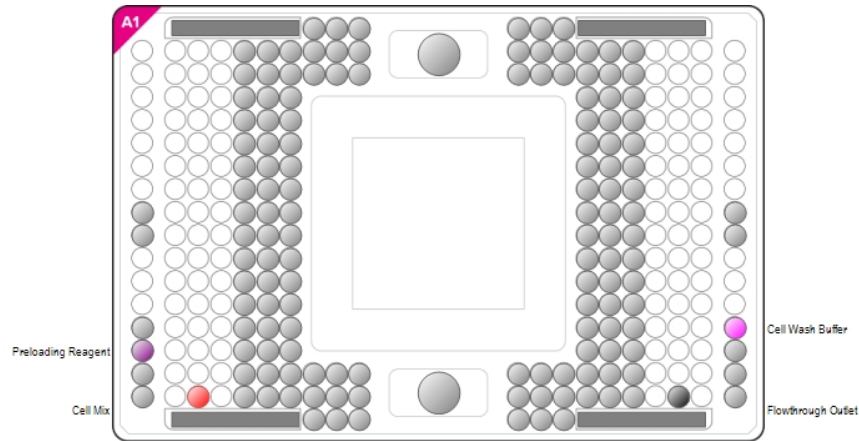
Script Reagent Details - Prime



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)			



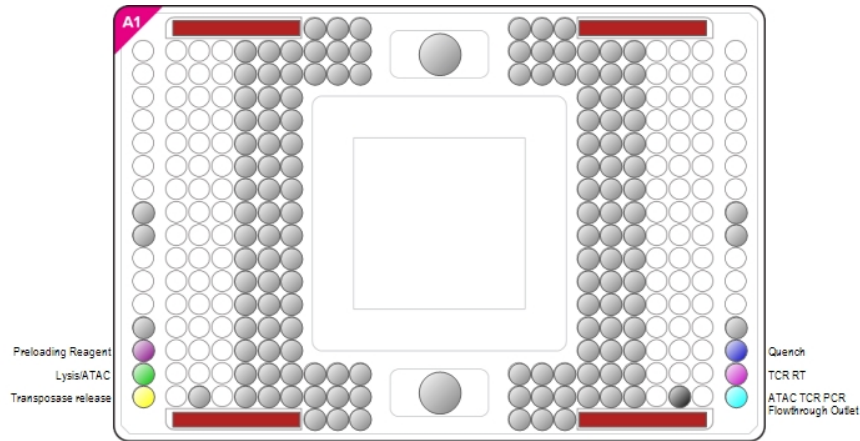
Script Reagent Details - Cell Load



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	5	
● 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 RGT (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 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	24	2	
● Lysis/ATAC	7	3	
● Transposase release	7	4	
● Quench	7	6	
● TCR RT	24	7	
● ATAC TCR PCR	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)			
Lysis/ATAC			
Reagent (Stock Concentration)	Mix Prep (µl)	Prep Conc.	Chamber Conc.
10% NP40 (10%)	0.45	0.15	0.1001
2X TD buffer (2X)	22.5	1.5	1.0005
20X Tn5 transposase (20X)	2.25	1.5	1.0005
20X C1 loading reagent low salt (20X)	2.25	1.5	1.0005
PCR grade water	0.3		

SuperaseIN RNase inhibitor (20 U/μl)	2.25	1.5	1.0005
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30 Total Prep Volume

Quench			
Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
50 mM MgCl ₂ (50 mM)	18	45	12.87
20X C1 loading reagent low salt (20X)	1	1	0.286
10 mM Tris-HCL pH 8.0 (10 mM)	1	0.5	0.143

20 Total Prep Volume

ATAC TCR PCR			
Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
20X C1 No salt loading reagent (20X)	1.5	1.0003	0.4481
PCR water	8.62		
5X NEB Q5 buffer (5X)	13.4	2.2341	1.0009
5mM dNTP (5 mM)	1.2	0.2001	0.0896
6uM TCR primer mix (6 μM)	3	0.6002	0.2689
2U/ul NEB Q5 DNA Polymerase (2 U/μl)	0.67	0.0447	0.02
25uM ATAC primer 1 (25 μM)	0.8	0.6669	0.2988
25 uM ATAC primer 2 (25 μM)	0.8	0.6669	0.2988

29.99 Total Prep Volume

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

Transposase release			
Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
500 mM EDTA (500 mM)	2	50	20
20X C1 loading reagent low salt (20X)	1	1	0.4
10 mM Tris-HCl, pH8 (10 mM)	17	8.5	3.4

20 Total Prep Volume

TCR RT			
Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
20X C1 loading reagent no salt (20X)	1.5	1	0.811
PCR water	15.55		
10X Sensiscript RT buffer (10X)	3.7	1.2333	1.0002
5mM dNTPs (5 mM)	3.7	0.6167	0.5001
6 uM TCR primer mix (6 μM)	3.7	0.74	0.6001
20X Sensiscript RT enzyme (20X)	1.85	1.2333	1.0002



30 Total Prep Volume



Protocol Reagent Shopping List

Reagent Name	Vendor	Part Number	Kit Part Number	Stock Concentration
10% NP40	Igepal	CA-630		10%
2X TD buffer	Illumina	15027987	15027987	2X
20X Tn5 transposase	Illumina	FC-121-1030		20X
SuperaseIN RNase inhibitor	Thermofisher	AM2694		20 U/μl
50 mM MgCl ₂	BioRad	1708872		50 mM
10 mM Tris-HCL pH 8.0	Thermofisher	15568025		10 mM
5X NEB Q5 buffer	NEB	B90275		5X
5mM dNTP	NEB	B90275		5 mM
2U/ul NEB Q5 DNA Polymerase	NEB	B90275		2 U/μl
500 mM EDTA	Thermofisher	15575020		500 mM
10 mM Tris-HCl, pH8	Thermofisher	15568025		10 mM
10X Sensiscript RT buffer	Qiagen	205213		10X
5mM dNTPs	Qiagen	205213		5 mM
20X Sensiscript RT enzyme	Qiagen	2025213		20X

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"/>		
20X C1 loading reagent low salt		20X					
20X C1 loading reagent no salt	100-8920	20X					