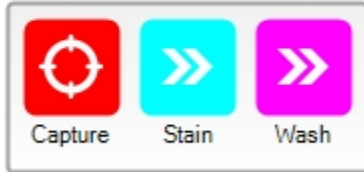




Name	ATAC Seq
Revision	C
Description	
Authors	Buenrostro et al.
Institution	Stanford University
Lab	William Greenleaf and Howard Chang
Special Instructions	Refer to attached file ATAC Seq Post C1 protocol for instructions on sequencing library preparation after C1 harvest.

Cell Load and Stain



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 and Stain

Runtime Estimates

Barcode	Estimate
1861x (5-10 um diameter cells)	0 hours, 31 minutes
1862x (10-17 um diameter cells)	1 hours, 0 minutes
1863x (17-25 um diameter cells)	0 hours, 51 minutes

Incubation Profile

Script Step	Operation	Temperature (C)	Duration (s)
Stain	Incubation	25	600

Script Summary - Sample Prep

Runtime Estimates

Barcode	Estimate
1861x (5-10 um diameter cells)	4 hours, 29 minutes
1862x (10-17 um diameter cells)	4 hours, 29 minutes
1863x (17-25 um diameter cells)	4 hours, 29 minutes

Incubation Profile

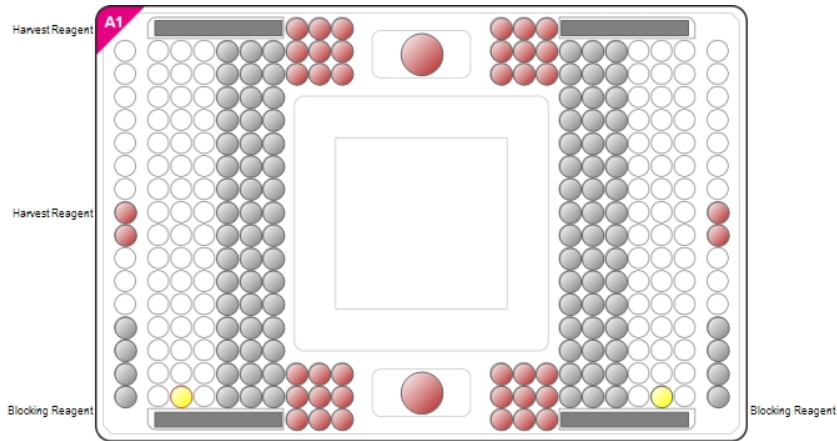
Script Step	Operation		Temperature (C)	Duration (s)
Lysis/ATAC	Incubation	S1	37	1800
EDTA	Incubation	S1	50	1800



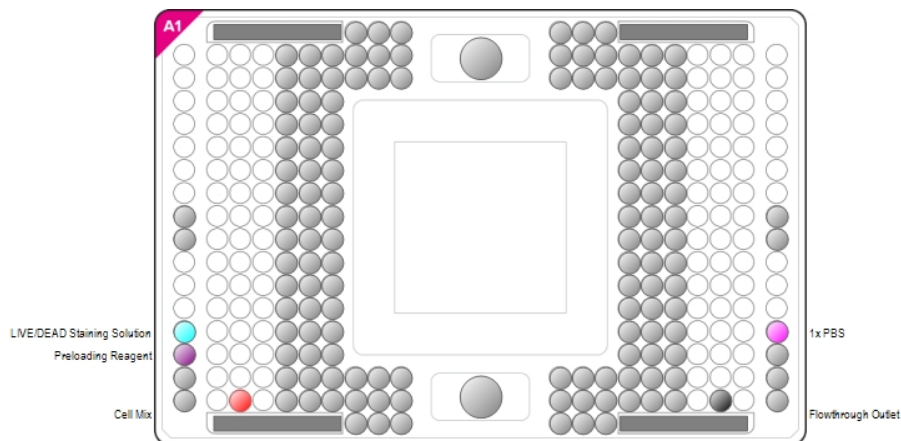
PCR	Extension	Filling Ends	72	300
PCR	PCR	Denaturation	98	30
PCR	PCR x8	Denaturation	98	10
		Annealling	72	30
		Extension	72	60



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)			

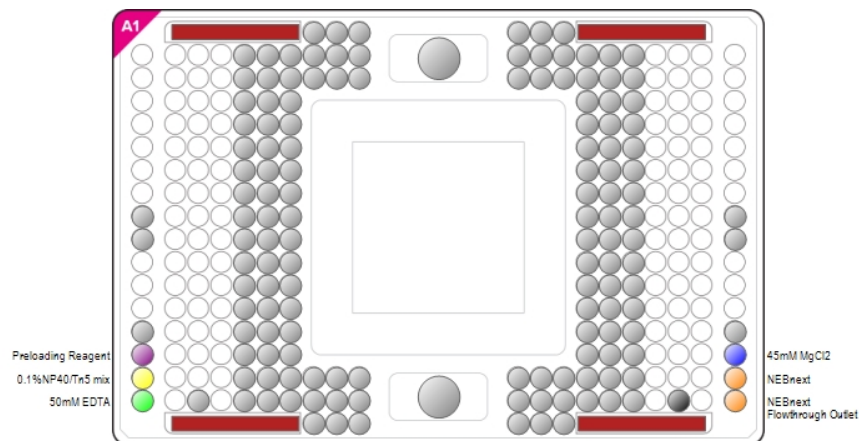
Script Reagent Details - Cell Load and Stain


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
● LIVE/DEAD Staining Solution	7	1	
● Preloading Reagent	24	2	
● 1x PBS	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 66-330 / µL	60		
100 Total Prep Volume			
LIVE/DEAD Staining Solution			
Reagent (Stock Concentration)	Mix Prep (µl)	Prep Conc.	Chamber Conc.
Cell Wash BUF (1X)	1250	0.9975	0.9975
Ethidium homodimer-1 (2 mM)	2.5	0.004	0.004
Calcein AM (4 mM)	0.625	0.002	0.002



1253.125 Total Prep Volume

1x PBS			
Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
1x PBS (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	
● 0.1%NP40/Tn5 mix	7	3	
● 50mM EDTA	7	4	
● 45mM MgCl2	7	6	
● NEBnext	24	7	
● NEBnext	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)			
0.1%NP40/Tn5 mix			
Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
10% NP40 (10%)	0.45	0.15	0.1001
2x TD (2X)	22.5	1.5	1.0005
Tn5 Transposase (20X)	2.25	1.5	1.0005
H2O	2.55		
C1 No Salt Loading RGT (20X)	2.25	1.5	1.0005

30 Total Prep Volume

50mM EDTA			
Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
EDTA (500 mM)	2	50	20
Tris-HCl, pH 8 (10 mM)	17	8.5	3.4
C1 No Salt Loading RGT (20X)	1	1	0.4

20 Total Prep Volume

NEBnext			
Comments: -----			
Primer 1: GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG			
Primer 2: TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG			
Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
NEBNext® High-Fidelity 2X PCR Master Mix (2X)	30.15	1.1165	0.9054
Primer 1 (25 μM)	3.02	1.3979	1.1337
Primer 2 (25 μM)	3.02	1.3979	1.1337
H2O	15.12		
C1 No Salt Loading RGT (20X)	2.7	0.9998	0.8108

54.01 Total Prep Volume

Harvest Reagent			
Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
C1 Harvest RGT (1X)			
45mM MgCl2			
Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
MgCl2 (50 mM)	27	45	12.87
Tris-HCl, pH 8 (10 mM)	1.5	0.5	0.143
C1 No Salt Loading RGT (20X)	1.5	1	0.286

30 Total Prep Volume

Protocol Reagent Shopping List

Reagent Name	Vendor	Part Number	Kit Part Number	Stock Concentration
Ethidium homodimer-1	Life Technologies		L-3224	2 mM
Calcein AM	Life Technologies		L-3224	4 mM
1x PBS	Life Technologies	10010-023		1X
10% NP40	Life Technologies		28324	10%
2x TD	Illumina		FC-121-1030	2X
Tn5 Transposase	Illumina		FC-121-1030	20X
H2O	Life Technologies		AM9937	
EDTA	Life Technologies		AM9260G	500 mM
Tris-HCl, pH 8	Life Technologies		AM9855G	10 mM
NEBNext® High-Fidelity 2X PCR Master Mix	NEB		M0541	2X
Primer 1	IDT			25 uM
Primer 2	IDT			25 uM
MgCl2	Life Technologies		AM9530G	50 mM

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 No Salt Loading RGT	101-0133	20X	<input checked="" type="checkbox"/>				