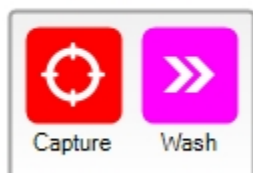


Name ATAC Seq
Revision C
Description
Authors Buenrostro et al.
Institution Stanford University
Lab William Greenleaf and Howard Chang
Special Instructions

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

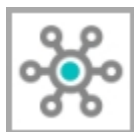
Incubation Profile

Script Step	Operation	Temperature (C)	Duration (s)
Lysis/ATAC	Incubation	37	1800
Release	Incubation	50	1800
PCR	FillingEnds	72C	300
PCR	Denaturation	98C	30
PCR	PCR x8	Denaturation	10
	Annealling	72	30

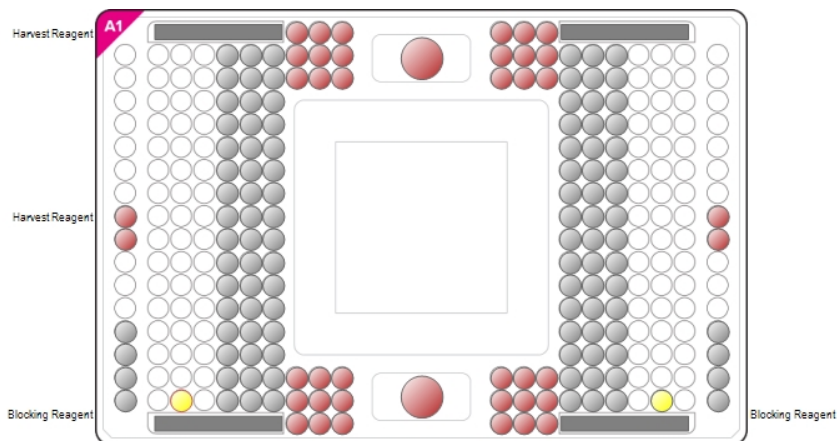
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Extension	72	60
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Script Reagent Details - Prime



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	

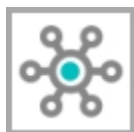
Reagent Mix Recipe - Prime

Blocking Reagent

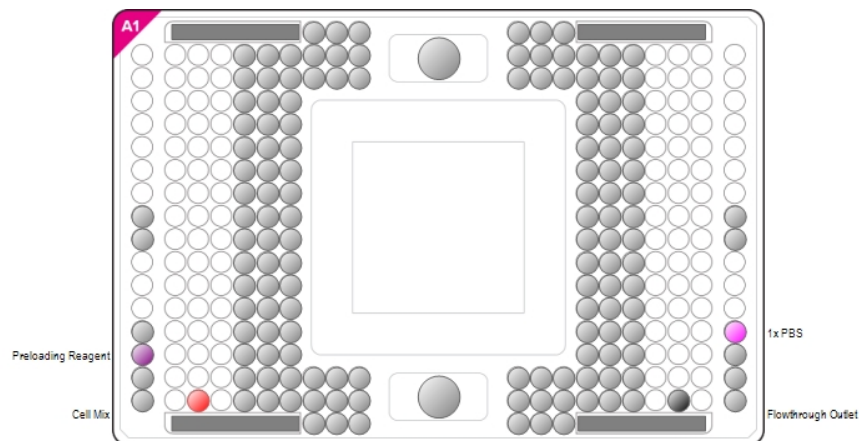
Reagent (Stock Concentration)	Mix Prep (µl)	Prep Conc.	Chamber Conc.
C1 Blocking RGT (1X)			

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	
● 1x PBS	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)			

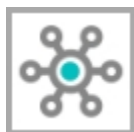
Cell Mix

Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
Suspension RGT (2.5X)	40	1	1
Cells 66-330 / μL	60		

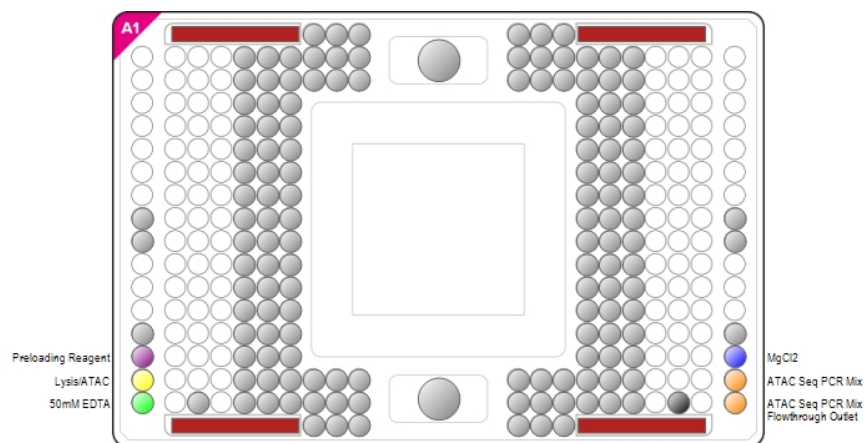
100 Total Prep Volume

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	
● Lysis/ATAC	7	3	
● 50mM EDTA	7	4	
● MgCl ₂	7	6	
● ATAC Seq PCR Mix	24	7	
● ATAC Seq PCR Mix	24	8	
● Harvest Reagent	180 μl each	Harvest Inlets	

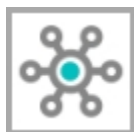
Reagent Mix Recipe - Sample Prep

Preloading Reagent

Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
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 (2X)	22.5	1.5	1.0005
Tn5 Transposase (20X)	2.25	1.5	1.0005
H ₂ O	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)	3	50	20
Tris-HCl, pH 8 (10 mM)	25.5	8.5	3.4
C1 No Salt Loading RGT (20X)	1.5	1	0.4

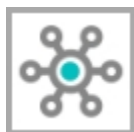
30 Total Prep Volume

ATAC Seq PCR Mix			
Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
NEBNext® High-Fidelity 2X PCR Master Mix (2X)	30.2	1.1185	0.9071
H2O	15.1		
Primer 1 (25 uM)	3	1.3889	1.1264
Primer 2 (25 uM)	3	1.3889	1.1264
C1 No Salt Loading RGT (20X)	2.7	1	0.811

54 Total Prep Volume

Harvest Reagent			
Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
C1 Harvest RGT (1X)			
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
10% NP40	Life Technologies		28324	10%
2x TD	Illumina		FC-121-1030	2X
Tn5 Transposase	Illumina		FC-121-1030	20X
EDTA	Life Technologies		AM9260G	500 mM
NEBNext® High-Fidelity 2X PCR Master Mix	NEB		M0541	2X

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"/>	
1x PBS	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"/>				