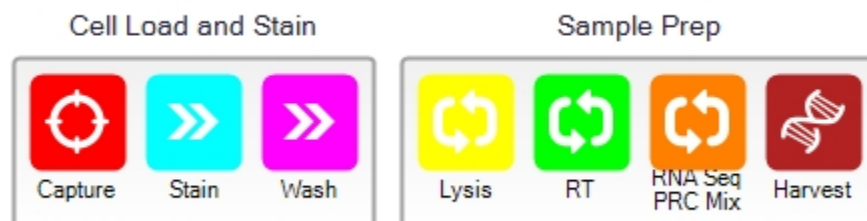


Name: CORTAD Seq
 Version: 1
 Description: Concurrent RNA and Targeted DNA sequencing
 Authors: Say Li Kong
 Institution: Genome Institute of Singapore
 Lab:
 Special Instructions:



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, 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

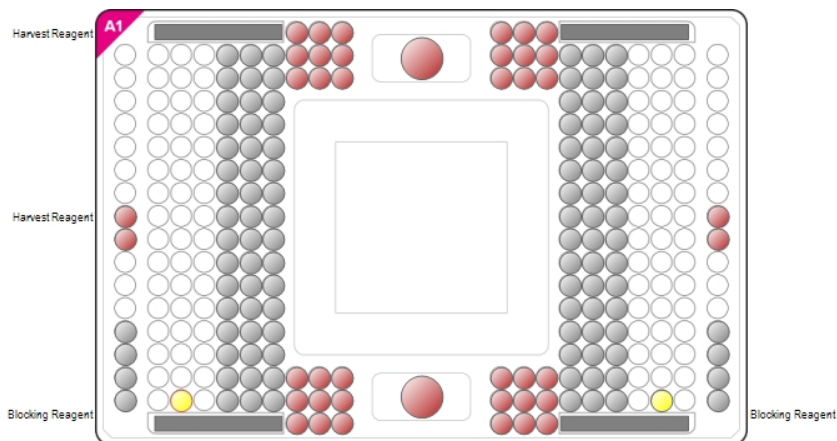
Runtime Estimates

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

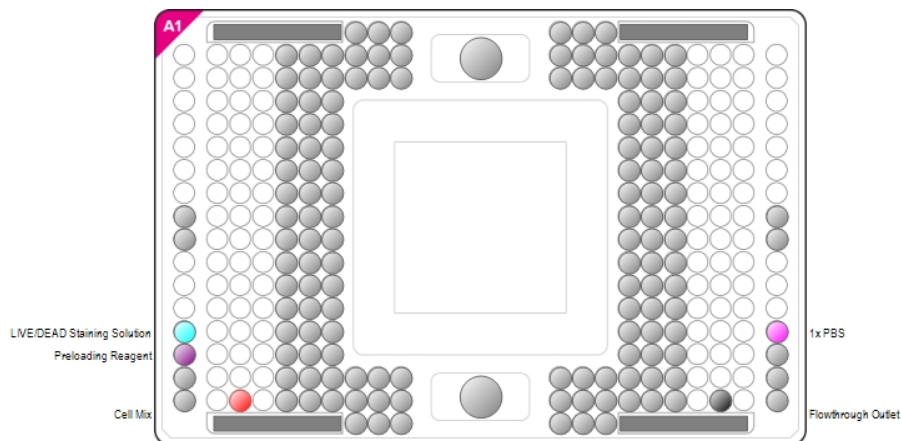
Incubation Profile

Script Step	Operation		Temperature (C)	Duration (s)
Lysis	Incubation	S1	37	600
		S2	72	180
		S3	4	600

		S4	25	60
RT	Incubation	S1	42	5400
		S2	70	600
RNA Seq PRC Mix	Hot Start	95C	95	60
RNA Seq PRC Mix	PCR x5	Denaturation	95	20
		Annealling	58	240
		Extension	68	360
RNA Seq PRC Mix	PCR x9	Denaturation	95	20
		Annealling	64	30
		Extension	68	360
RNA Seq PRC Mix	PCR x7	Denaturation	95	30
		Annealling	64	30
		Extension	68	420
RNA Seq PRC Mix	Extension	72C	72	600

Script Reagent Details - Prime


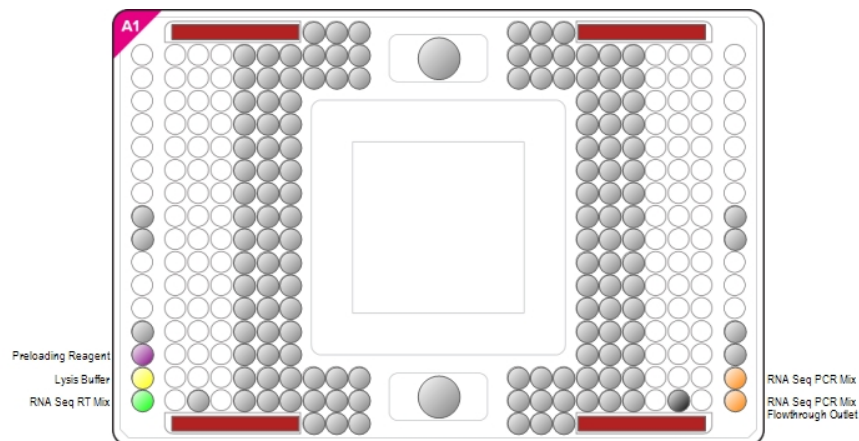
Reagent Loading			
Name	Volume (µl)	IFC Inlet	Notes
● Harvest Reagent	200 µl	A1	
● Harvest Reagent	200 µl	A2	
● Blocking Reagent	15 µl	C1	
● Blocking Reagent	15 µl	C2	
● Harvest Reagent	20 µl	P1	
● Harvest Reagent	20 µl	P2	
Reagent Mix Recipe - Prime			
Blocking Reagent			
Reagent (Stock Concentration)	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 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 166-330 / 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		
● Lysis Buffer	7	3		
● RNA Seq RT Mix	8	4		
● RNA Seq PCR Mix	24	7		
● RNA 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)				
Cell Lysis Buffer (Secondary)				
Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.	
Triton X-100 (1.5%)				
TRIS pH8.0 (100 mM)				
KCl (100 mM)				
EDTA (5 mM)				
Lysis Buffer				
Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.	

C1 Loading RGT (20X)	3	1.4252	0.9506
RNase Inhibitor (40 U/μl)	1	0.9501	0.6337
3' SMART CDS Primer IIA (12 μM)	14	3.9905	2.6617
Water (1X)	11.5	0.2732	0.1822
Polaris Lysis plus reagent (1X)	0.6	0.0143	0.0095
Cell Lysis Buffer	12		

42.1 Total Prep Volume

RNA Seq RT Mix			
Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
C1 Loading RGT (20X)	1.2	0.7229	0.4128
5X First-Strand Buffer (Rnase-free) (5X)	11.2	1.6867	0.9631
Dithiothreitol (100 mM)	1.4	4.2169	2.4078
dNTP Mix (10 mM)	5.6	1.6867	0.9631
SMARTer IIA Oligonucleotide (12 μM)	5.6	2.0241	1.1558
RNase Inhibitor (40 U/μl)	1.4	1.6867	0.9631
SMARTScribe Reverse Transcriptase (100 U/μl)	5.6	16.8675	9.6313
Polaris RT plus reagent (20X)	1.2	0.7229	0.4128

33.2 Total Prep Volume

RNA Seq PCR Mix			
Comments: -----			
3 sets of EGFR gDNA primers: Three sets of EGFR gDNA primers (4.5 μM, 1 uL primer set)			
Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
C1 Loading RGT (20X)	4.5	1	0.811
PCR-Grade Water	60.5		
10X Advantage 2 PCR Buffer (10X)	10	1.1111	0.9011
50X dNTP Mix (10 mM)	4	0.4444	0.3604
IS PCR primer (12 μM)	4	0.5333	0.4325
50X Advantage 2 Polymerase Mix (50X)	4	2.2222	1.8022
3 sets of EGFR gDNA primers (4.5 μM)	3	0.15	0.1217

90 Total Prep Volume

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

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
RNase Inhibitor	Clontech		634833	40 U/μl
3' SMART CDS Primer IIA	Clontech		634833	12 μM
Water	Clontech			1X
Triton X-100	BioRad	161-0407		1.5%
TRIS pH8.0	Ambion	AM9855G		100 mM
KCl	1st Base	BUF-1310-100ML		100 mM
EDTA	Ambion	AM9260G		5 mM
5X First-Strand Buffer (Rnase-free)	Clontech		634833	5X
Dithiothreitol	Clontech		634833	100 mM
dNTP Mix	Clontech		634833	10 mM
SMARTer IIA Oligonucleotide	Clontech		634833	12 μM
SMARTScribe Reverse Transcriptase	Clontech		634833	100 U/μl
PCR-Grade Water	Clontech		639207	
10X Advantage 2 PCR Buffer	Clontech		639207	10X
50X dNTP Mix	Clontech		639207	10 mM
IS PCR primer	Clontech		634833	12 μM
50X Advantage 2 Polymerase Mix	Clontech		639207	50X

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"/>