

Name C1-RamDA-seq
 Revision A
 Description Gene expression - total RNA sequencing
 Authors Tetsutaro Hayashi, Mana Umeda, Haruka Ozaki and Itoshi Nikaido
 Institution Laboratory for Bioinformatics Research
 Lab RIKEN Center for Biosystems Dynamics Research
 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, 52 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)	6 hours, 58 minutes
1862x (10-17 um diameter cells)	6 hours, 58 minutes
1863x (17-25 um diameter cells)	6 hours, 58 minutes

Incubation Profile

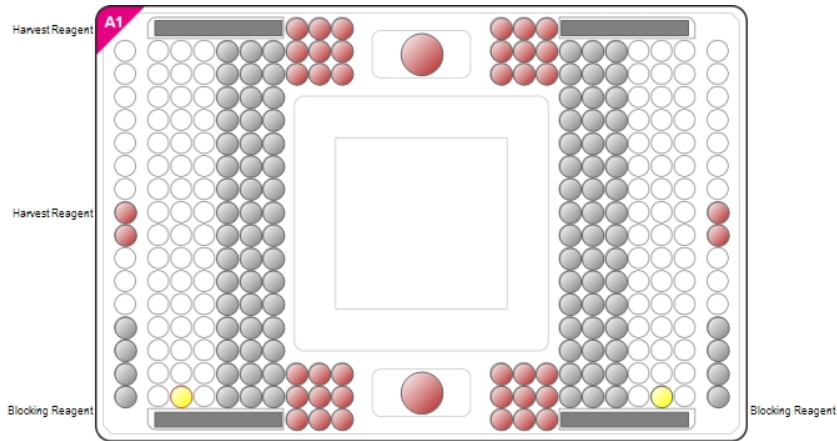
Script Step	Operation	Temperature (C)	Duration (s)
Lysis	Denature	incubation	4
		Denaturation	70
		Incubation	4
			1
			90
			300



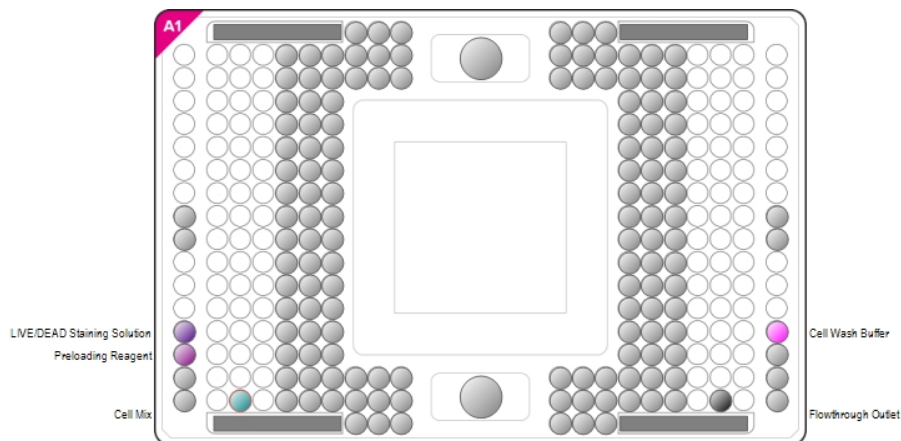
gDNA digestion	gDNA Digestion	gDNA Digestion	30	300
		Incubation	4	1
Priming	Priming	Prime1	25	600
		Prime2	30	600
RT-RamDA	RT-RamDA II	RT-1	37	2700
		RT-2	50	300
		Denaturation	94	300
		Incubation	4	1
2nd Strand Synthesis	2nd Strand Synthesis	2nd Strand Synthesis	16	3600
		Denaturation	75	1200



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			

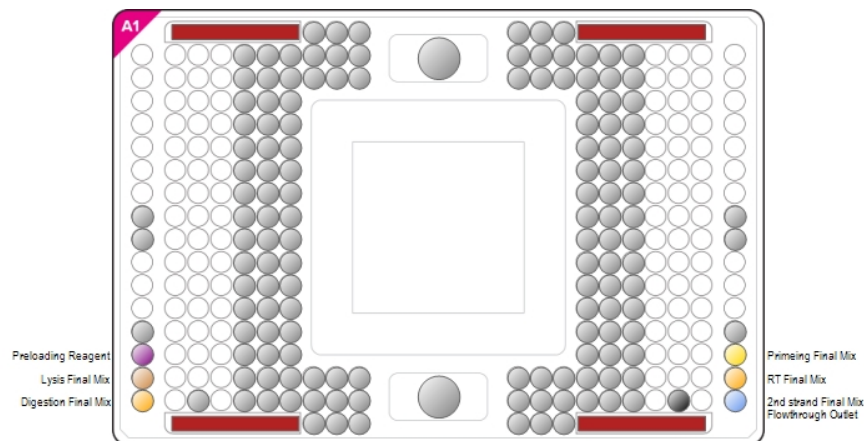
Script Reagent Details - Cell Load and Stain


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	↻ 20	2	
● Cell Wash Buffer	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.
Not Defined			
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

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		
● Digestion Final Mix	7	4		
● Primeing Final Mix	7	6		
● RT Final Mix	24	7		
● 2nd strand Final 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.	
Not Defined				
30 X RT Primer mix (Secondary: 30X)				
Special Instructions:				

For 100 uM 1st-NSR primers, please refer to the attached table "List of not-so-random primers" and order the NSR primers to custom oligo synthesis services. Mix all oligos at the equal volume at 100 uM.				
Comments:				

30 uM Oligo(dT)18 Primer: Dilute the Oligo(dT)18 Primer to 30 uM with RNase-free water				
Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.	

30 uM Oligo(dT)18 Primer (30 uM)	2	6
100 uM 1st-NSR primers (100 μM)	8	80

10 Total Prep Volume

Primeing Final Mix

Comments:

 T4 Gene 32 Protein : T4 gene 32 protein (10 mg/ml) must be diluted to 2 mg/mL with RNase-free water just before use

Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
5 X PrimeScript RT buffer (5X)	17.23	2.8717	0.8213
20 X PrimeScript RT Enzyme Mix (20X)	5.24	3.4933	0.9991
T4 Gene 32 Protein (2 mg/ml)	1.75	0.1167	0.0334
30 X RT Primer mix (30X)	3.5	3.5	1.001
RNase-free Water	1.15		
C1 Loading RGT (20X)	1.13	0.7533	0.2155

30 Total Prep Volume

2nd strand Final Mix

Special Instructions:

 For 100 uM 2nd-NSR primers, please refer to the attached table "List of not-so-random primers" and order the NSR primers to custom oligo synthesis services. Mix all oligos at the equal volume at 100 uM.

Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
10 X NEB buffer 2 (10X)	6.7	2.2333	1.0005
dNTP Mixture (2.5 mM each) (2.5 mM)	6.7	0.5583	0.2501
100 uM 2nd-NSR primers (100 μM)	5.36	17.8667	8.0043
Klenow Fragment (3'-5' exo-) (5 U/μl)	2.01	0.335	0.1501
RNase-free Water	7.73		
C1 Loading RGT (20X)	1.5	1	0.448

30 Total Prep Volume

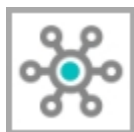
Harvest Reagent

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

Lysis Final Mix

Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
NP-40 Surfact-Amps Detergent Solution 10% (w/v) (10 %)	1.21	0.4481	0.2989
Roche Cell Lysis buffer (bottle 1) (100%)	4.05	15	10.005
RNasin Plus RNase Inhibitor 40U/ul (40 U/μl)	0.84	1.2444	0.83
1/5000 ERCC RNA Spike-In Mix (13.5X)	3	1.5	1.0005
RNase-free Water	16.55		

9/17/2018 6:17:28 PM



C1 Loading RGT (20X)	1.35	1	0.667
----------------------	------	---	-------

27 Total Prep Volume

Digestion Final Mix

Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
5 x PrimeScript RT buffer (5X)	2.5	0.625	0.25
DNase I, Amplification Grade (1 U/μl)	5	0.25	0.1
RNase-free Water	11.5		
C1 Loading RGT (20X)	1	1	0.4

20 Total Prep Volume

RT Final Mix

Comments:

T4 Gene 32 Protein: T4 gene 32 protein (10 mg/ml) must be diluted to 2 mg/mL with RNase-free water just before use

Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
RNase-free Water	33.92		
C1 Loading RGT (20X)	2.25	0.75	0.6083
T4 Gene 32 Protein (2 mg/ml)	1	0.0333	0.027
20 X PrimeScript RT Enzyme Mix (20X)	3	1	0.811
5 X PrimeScript RT buffer (5X)	12	1	0.811
DNase I, Amplification Grade (1 U/μl)	3.96	0.066	0.0535
Roche Cell Lysis buffer (bottle 1) (100%)	1.87	3.1167	2.5276
30 X RT Primer mix (30X)	2	1	0.811

60 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
5 X PrimeScript RT buffer	TaKaRa		RR037A	5X
20 X PrimeScript RT Enzyme Mix	TaKaRa		RR037A	20X
T4 Gene 32 Protein	NEB	M0300L		2 mg/ml
30 uM Oligo(dT)18 Primer	Thermo Fisher	# SO131		30 uM
RNase-free Water	TaKaRa		RR037A	
10 X NEB buffer 2	NEB		M0212L	10X
dNTP Mixture (2.5 mM each)	TaKaRa	4030		2.5 mM
Klenow Fragment (3'-5' exo-)	NEB		M0212L	5 U/μl
NP-40 Surfact-Amps Detergent Solution 10% (w/v)	Thermo Fisher	28324		10%
Roche Cell Lysis buffer (bottle 1)	Roche		0636682100 1	100%
RNasin Plus RNase Inhibitor 40U/ul	Promega	N2611		40 U/μl
1/5000 ERCC RNA Spike-In Mix	Life Technologies	4456740		13.5X
DNase I, Amplification Grade	Life Technologies	18068-015		1 U/μl
T4 Gene 32 Protein	NEB	M0300L		2 mg/ml
DNase I, Amplification Grade	Life Technologies	18068-015		1 U/μl

Fluidigm Reagent Kits

Reagent Name	Part Number	Stock Concentration	PN 100-8920	PN 100-6201	PN 100-5319	PN 100-7357	PN 100-8921
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"/>
C1 Harvest RGT	100-6248	1X	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>		<input checked="" type="checkbox"/>	