



Name	Mini-metagenomics
Revision	A
Description	Lysozyme and MDA on the C1 chip using Qiagen Repli-g sc
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Special Instructions	Nuclease free water, Tris-Cl, and DBL2 solution should be UV treated for 30 minutes before use. Tween 20 should be diluted to a final concentration of 10% in UV treated water.



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, 12 minutes
1863x (17-25 um diameter cells)	0 hours, 12 minutes

Script Summary - Random Capture

Runtime Estimates

Barcode	Estimate
1861x (5-10 um diameter cells)	0 hours, 16 minutes
1862x (10-17 um diameter cells)	0 hours, 26 minutes
1863x (17-25 um diameter cells)	0 hours, 25 minutes

Script Summary - Qiagen Lysis MDA

Runtime Estimates

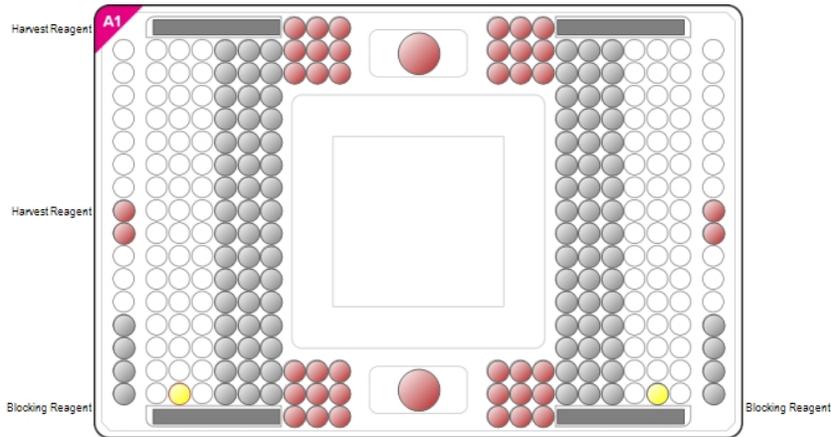
Barcode	Estimate
1861x (5-10 um diameter cells)	7 hours, 23 minutes
1862x (10-17 um diameter cells)	7 hours, 23 minutes
1863x (17-25 um diameter cells)	7 hours, 23 minutes

Incubation Profile

Script Step	Operation	Temperature (C)	Duration (s)
Lysozyme	Incubation	37	1800
MDA	MDA	Amplification	30
		Inactivation	70



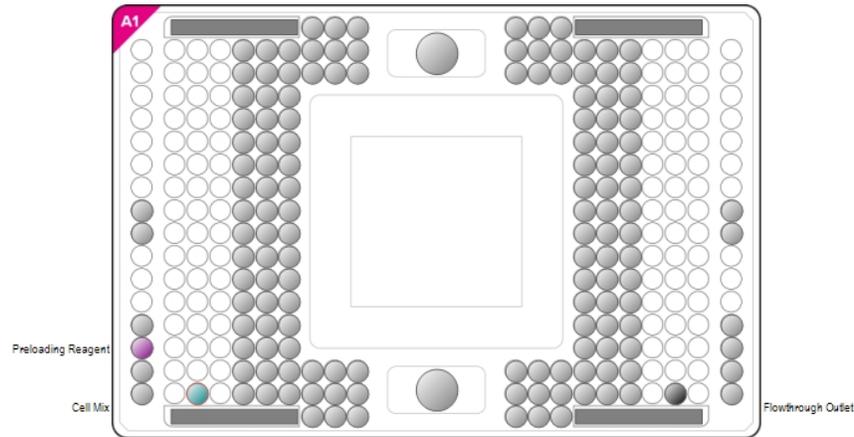
Script Reagent Details - Prime



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)			



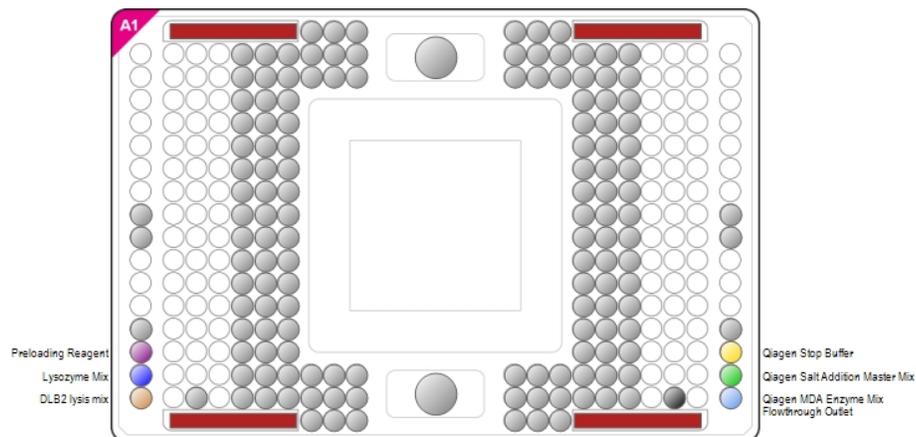
Script Reagent Details - Random Capture



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	20	2	
● Cell Mix	10	C1	
Reagent Mix Recipe - Random Capture			
Preloading Reagent			
Reagent (Stock Concentration)	Mix Prep (µl)	Prep Conc.	Chamber Conc.
C1 Preloading RGT (1X)			
Cell Mix			
Special Instructions:			

SAMPLE PREPARATION			
Centrifuged quickly for the dirt to settle in the tube (typically using 2 mL tube)			
Transfer clear sample into a clean tube			
Spin down the sample at 5000g for 7 min			
Remove the supernatant and resuspend the cells in UV treated 1% NaCl			
Dilute to ~2 cells per nL (2×10^6 cells / mL) in UV treated 1% NaCl.			
Load 15 uL into cell loading port.			
Reagent (Stock Concentration)	Mix Prep (µl)	Prep Conc.	Chamber Conc.
Cells ~2 Million/mL	20		

20 Total Prep Volume

Script Reagent Details - Qiagen Lysis MDA


Inlet Reuse			
Name	IFC Inlet	Instructions	
● Preloading Reagent	2	Aspirate inlet prior to loading reagents	
Reagent Loading			
Name	Volume (μl)	IFC Inlet	Notes
● Preloading Reagent	20	2	
● Lysozyme Mix	7	3	
● DLB2 lysis mix	7	4	
● Qiagen Stop Buffer	9	6	
● Qiagen Salt Addition Master Mix	24	7	
● Qiagen MDA Enzyme Mix	24	8	
● Harvest Reagent	180 μl each	Harvest Inlets	
Reagent Mix Recipe - Qiagen Lysis MDA			
Preloading Reagent			
Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
C1 Preloading RGT (1X)			
DLB2 solution (Secondary: 1X)			
Special Instructions:			

This is the same thing as Buffer D2 in the Qiagen repli-g sc protocol. DLB2 is UV treated for 30 min after preparation.			
Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
DLB Buffer (1X)	33	0.9167	
DTT	3		

36 Total Prep Volume

DLB2 lysis mix			
Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
Tween 20 (10%)	1	0.5	0.2
DLB2 solution (1X)	19	0.95	0.38

20 Total Prep Volume

Qiagen Stop Buffer			
Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
Repli-g stop solution (1X)	19	0.95	0.2717
Tween 20 (10%)	1	0.5	0.143

20 Total Prep Volume

Qiagen MDA Enzyme Mix			
Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
Repli-g Single Cell Polymerase (1X)	3	0.0889	0.0398
Repli-g Single Cell Reaction Buffer (1X)	30.75	0.9111	0.4082

33.75 Total Prep Volume

Harvest Reagent			
Special Instructions: ----- Samples do not need to be diluted prior to measuring DNA concentration			

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

Lysozyme Mix			
Special Instructions: ----- Tris-Cl should be UV be UV treated for 30 min before use. Tween 20 should be diluted to a final concentration of 10% in UV treated water. Ready-Lyse should be diluted to a final concentration of 400 U/μL in UV treated 10 mM Tris-Cl.			

Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
UltraPure Tris-Cl (10 mM)	7.5	1.5	1.0005
Tween 20 (10%)	21.2	4.24	2.8281
Epicenter Ready-Lyse (400 U/μl)	21.3	170.4	113.6568

50 Total Prep Volume



Qiagen Salt Addition Master Mix

Comments:

nuclease free water: UV treat for 30 min

Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
nuclease free water (1X)	16.25	0.4815	0.3905
Repli-g single cell reaction buffer (1X)	13	0.3852	0.3124
Repli-g stop solution (1X)	2.25	0.0667	0.0541
DLB2 solution (1X)	2.25	0.0667	0.0541

33.75 Total Prep Volume

Protocol Reagent Shopping List

Reagent Name	Vendor	Part Number	Kit Part Number	Stock Concentration
Tween 20	Sigma			10%
DLB Buffer	Qiagen			1X
DTT	Qiagen			
Repli-g stop solution	Qiagen			1X
Repli-g Single Cell Polymerase	Qiagen			1X
Repli-g Single Cell Reaction Buffer	Qiagen			1X
UltraPure Tris-Cl	Invitrogen			10 mM
Epicenter Ready-Lyse	Epicenter			400 U/μl

Fluidigm Reagent Kits

Reagent Name	Part Number	Stock Concentration	PN 100-8920	PN 100-6201	PN 100-7357	PN 100-5319	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"/>	