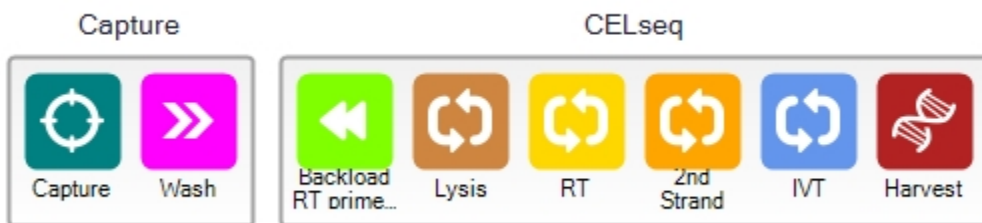


Name: CEL Seq v1
 Revision: B
 Description: 3'-end counting mRNAseq method that uses in vitro transcription for initial amplification. Adapted from Hashimshony, Wagner, Sher, & Yanai (2012) "CEL-Seq: Single-Cell RNA-Seq by Multiplexed Linear Amplification," Cell Reports 2:666-673.
 Authors: Tamar Hashimshony and Itai Yanai
 Institution: Technion - Israel Institute of Technology
 Lab: Itai Yanai
 Special Instructions: The sequences of the 96 reverse transcriptase primers are in attached file CelSeq_Primers.pdf. The protocol for processing the samples after the C1 is in attached file CEL-Seq Post C1 Processing Steps.pdf. Representative results showing comparability to the manual method are in attached file CEL-Seq Results on C1.pdf



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 - Capture

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 - CELseq

Runtime Estimates	
Barcode	Estimate
1861x (5-10 um diameter cells)	19 hours, 55 minutes
1862x (10-17 um diameter cells)	19 hours, 55 minutes
1863x (17-25 um diameter cells)	19 hours, 55 minutes

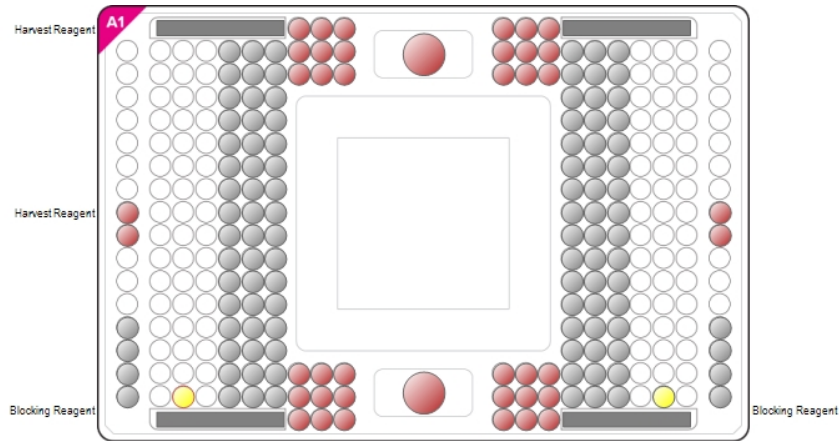
Incubation Profile			
Script Step	Operation	Temperature (C)	Duration (s)
Backload RT primers	Incubation	25	300
Lysis	Lysis	Lysis	300
		Cooling	60



RT	Reverse Transcriptase Reaction	Incubation	42	7200
		Cooling	10	60
2nd Strand	2nd Strand Synthesis	Incubation	16	7200
		Enzyme Inactivation	65	1200
		Cooling	10	60
IVT	T7 Transcription x12	Incubation	37	3600
IVT	Cooling	Cooling	10	60



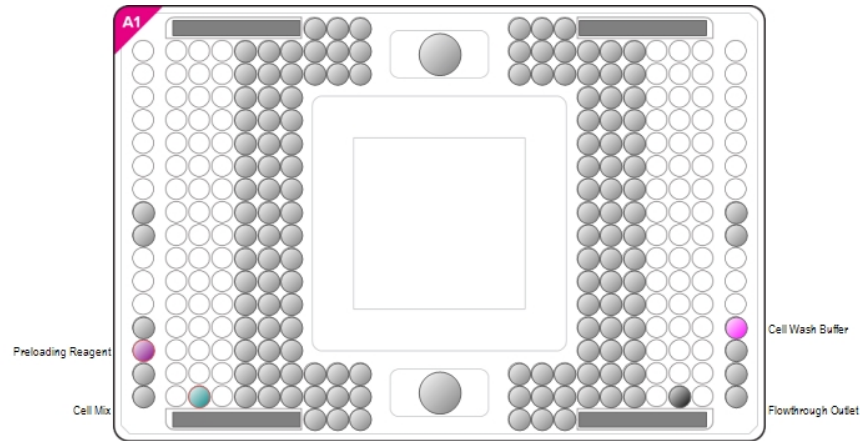
Script Reagent Details - Prime



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)			



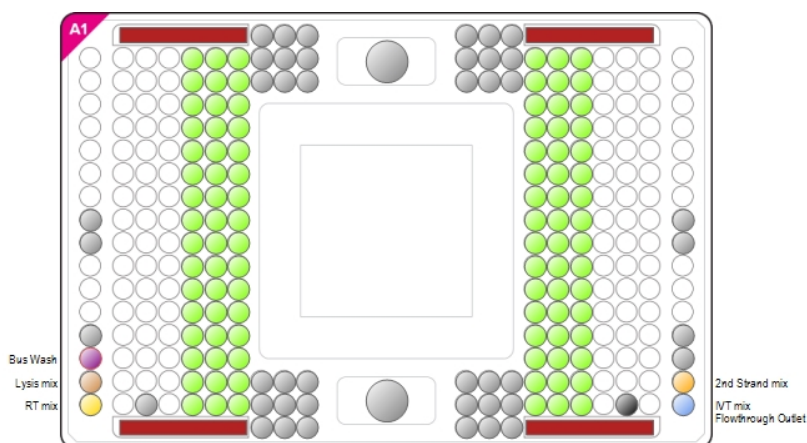
Script Reagent Details - Capture



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 Wash Buffer	7	5	
● Cell Mix	6	C1	
Reagent Mix Recipe - Capture			
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.
C1 Suspension RGT (2.5X)	40	1	1
Cells 66-330 / µL	60		
100 Total Prep Volume			
Cell Wash Buffer			
Reagent (Stock Concentration)	Mix Prep (µl)	Prep Conc.	Chamber Conc.
C1 Cell Wash BUF (1X)			

Script Reagent Details - CELseq

Sample Prep Description



Inlet Reuse			
Name	IFC Inlet	Instructions	
● Bus Wash	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
● Bus Wash	20	2	
● Lysis mix	7	3	
● RT mix	7	4	
● 2nd Strand mix	24	7	
● IVT mix	24	8	
● Harvest Reagent	180 μl each	Harvest Inlets	
● CEL-Seq primers	5 each	Harvest Outlets	
Reagent Mix Recipe - CELseq			
Bus Wash			
Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
Not Defined			
5X Lysis Buffer (Secondary: 5X)			
Special Instructions:			

Can be made up as a 100 ml stock by using ml in the recipe rather than μl. Store at room temperature.			
Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
NP-40 (10%)	25	2.5	
Tris-HCl, pH 8.4 (1000 mM)	25	250	

EDTA (500 mM)	1	5
Water	49	

100 Total Prep Volume

Lysis mix

Special Instructions:

1 ml is made so there is enough Lysis Mix to dilute the CEL-Seq primers (see previous step).

Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
SUPERase In (20 U/μl)	7	0.14	0.056
C1 Loading Reagent (20X)	70	1.4	0.56
Water	637.5		
1:100 dilution of ERCC RNA Spike-In Mix (1%)	5.5	0.0055	0.0022
5X Lysis Buffer (5X)	280	1.4	0.56

1000 Total Prep Volume

RT mix

Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
First Strand Buffer (10X)	7	3.5	1.001
dNTP (each) (25 mM)	1.4	1.75	0.5005
RNase Inhibitor (20X)	3.5	3.5	1.001
Array Script Reverse Transcriptase (20X)	3.5	3.5	1.001
C1 Loading Reagent (20X)	1	1	0.286
Water	3.6		

20 Total Prep Volume

25 mM each rNTP (Secondary: 25 mM)

Special Instructions:

Store at -20 degrees

Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
rATP (100 mM)	100	25	
rCTP (100 mM)	100	25	
rGTP (100 mM)	100	25	
rUTP (100 mM)	100	25	

400 Total Prep Volume

IVT mix

Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
T7 Reaction Buffer (10X)	7.5	2.2321	1
T7 Enzyme Mix (10X)	7.5	2.2321	1
C1 Loading Reagent (20X)	1.7	1.0119	0.4533

Water	13.9		
25 mM each rNTP (25 mM)	3	2.2321	1

33.6 Total Prep Volume

Harvest Reagent			
Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
C1 Harvest Reagent (1X)	180	1	

180 Total Prep Volume

CEL-Seq primers			
Special Instructions: -----			
Each CEL-Seq primer is one of 96, each with its own sample barcode. Sequences are in attached file CelSeq_Primers.pdf. These primer stocks should be stored in a 96-well plate at -20 degrees. The sequences of the primers are in the file CelSeq_Primers.pdf attached as part of the Script Hub submission. The process is to dispense 8 μl Lysis Mix (see Step: Lysis) to each well of a 96 -well plate. Then 2 μl primer is added to each well from the primer stock plate using an 8-channel pipettor. The 96-well plate with Lysis Mix plus primers is then gently vortexed and briefly centrifuged.			

Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
Lysis Mix (1X)	8	0.8	0.5336
CEL-Seq Primer (25 μg/ml)	2	5	3.335

10 Total Prep Volume

2nd Strand mix			
Reagent (Stock Concentration)	Mix Prep (μl)	Prep Conc.	Chamber Conc.
Second Strand Buffer (10X)	5	1.2346	1.0012
dNTP Mix (25X)	2	1.2346	1.0012
DNA Polymerase (50X)	1	1.2346	1.0012
RNase H (100X)	0.5	1.2346	1.0012
C1 Loading Reagent (20X)	2	0.9877	0.801
Water	30		

40.5 Total Prep Volume

Protocol Reagent Shopping List

Reagent Name	Vendor	Part Number	Kit Part Number	Stock Concentration
SUPERase In	Ambion	AM2694		20 U/μl
1:100 dilution of ERCC RNA Spike-In Mix	Ambion	4456740		1%
NP-40	Thermo Scientific	28324		10%
Tris-HCl, pH 8.4	Teknova	T1084		1000 mM
EDTA	Teknova	E0306		500 mM
First Strand Buffer	Life Technologies		AM1751	10X
dNTP (each)	Thermo Scientific	FERR1121		25 mM
RNase Inhibitor	Life Technologies		AM1751	20X
Array Script Reverse Transcriptase	Life Technologies		AM1751	20X
T7 Reaction Buffer	Life Technologies		AM1751	10X
T7 Enzyme Mix	Life Technologies		AM1751	10X
rATP	Promega	E6000		100 mM
rCTP	Promega	E6000		100 mM
rGTP	Promega	E6000		100 mM
rUTP	Promega	E6000		100 mM
Second Strand Buffer	Life Technologies		AM1751	10X
dNTP Mix	Life Technologies		AM1751	25X
DNA Polymerase	Life Technologies		AM1751	50X
RNase H	Life Technologies		AM1751	100X

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"/>	
C1 Suspension RGT	100-5315	2.5X	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	
C1 Cell Wash BUF	100-5314	1X	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>		
C1 Loading Reagent	100-5170	20X	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>			<input checked="" type="checkbox"/>
C1 Harvest Reagent	100-7081	1X					