Name	CEL Seq 2
Revision	A
Description	CELseq2 is a 3'-end counting mRNAseq method that uses in vitro transcription for initial amplification. CELseq2 incorporates numerous improvements over the original method including increased sensitivity and incorporation of unique molecular identif
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Institution	Technion - Israel Institute of Technology
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Special Instructions	T. Hashimshony, T. N. Senderovich, G. Avital, A. Klochendler, Y. de Leeuw, L. Anavy, D. Gennery, S. Li, K. J. Livak, O. Rozenblatt-Rosen, Y Dor, A. Regev, and I. Yanai. CEL-Seq2: sensitive highly-multiplexed single-cell RNA-Seq. Genome Biol 17:77 (2016). Complete protocol including sequences of all primers and post-C1 processing procedure are in the document entitled "CELSeq2 Protocol for Script Hub"

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

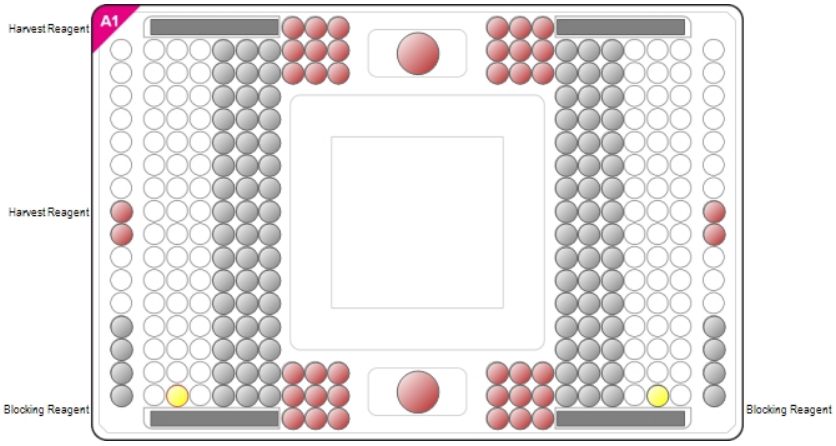
Barcode	Estimate
1861x (5-10 um diameter cells)	19 hours, 55 minutes
1862x (10-17 um diameter cells)	19 hours, 55 minutes
1863x (17-25 um diameter cells)	19 hours, 55 minutes

Incubation Profile

Script Step	Operation		Temperature (C)	Duration (s)
Backload RT primers	Incubation		25	300
Lysis	Lysis	Lysis	65	300

		Cooling	10	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

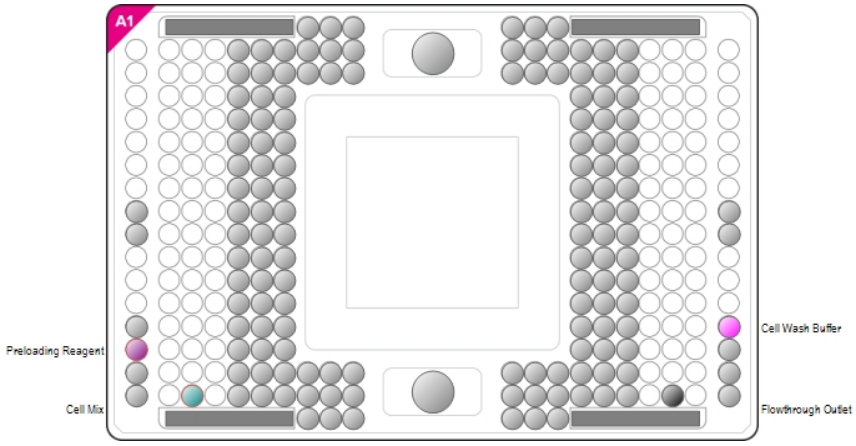
Blocking Reagent

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

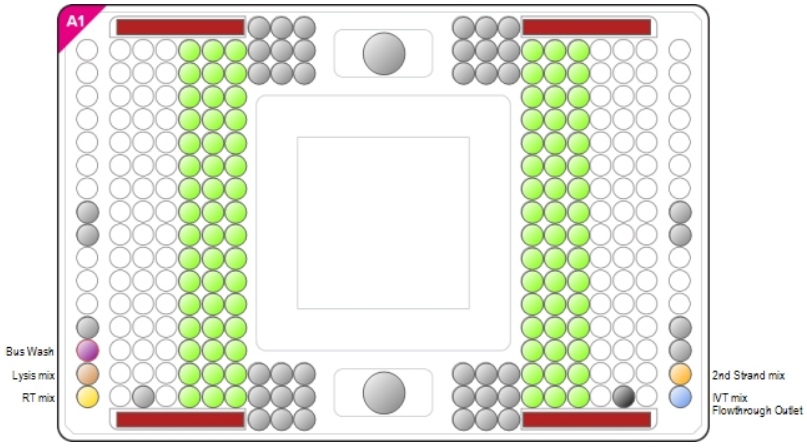
Reagent (Stock Concentration)	Mix Prep (µl)	Prep Conc.	Chamber Conc.
C1 Suspension RGT (2.5X)	40	1	1
Cells 66-330 / µL	60		
	100 Total Prep Volume		

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-Seq2 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:
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1 ml is made so there is enough Lysis Mix to dilute the CEL-Seq2 primers (see previous step).

Reagent (Stock Concentration)	Mix Prep (µl)	Prep Conc.	Chamber Conc.
RNaseOUT (40 U/µl)	4	0.16	0.064
C1 Loading Reagent (20X)	70	1.4	0.56
Water	610		
1:10,000 dilution of ERCC RNA Spike-In Mix (0.01%)	8	0.0001	0
5X Lysis Buffer (5X)	280	1.4	0.56
DTT (dithiothreitol) (1000)	28	28	11.2
	1000 Total Prep Volume		

RT mix

Comments:

First Strand Buffer: included with SuperScript II

Reagent (Stock Concentration)	Mix Prep (µl)	Prep Conc.	Chamber Conc.
First Strand Buffer (5X)	20	3.4965	1
dNTP Mix (each) (25 mM)	2	1.7483	0.5
RNaseOUT (40 U/µl)	2.6	3.6364	1.04
SuperScript II Reverse Transcriptase (200 U/µl)	2.6	18.1818	5.2
C1 Loading Reagent (20X)	1.4	0.979	0.28
	28.6 Total Prep Volume		

IVT mix

Reagent (Stock Concentration)	Mix Prep (µl)	Prep Conc.	Chamber Conc.
T7 Reaction Buffer (10X)	6.6	2.2297	0.9989
T7 Enzyme Mix (10X)	6.6	2.2297	0.9989
C1 Loading Reagent (20X)	1.5	1.0135	0.4541
rNTP Mix (each) (25 mM)	14.9	12.5845	5.6378
	29.6 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-Seq2 primers

Special Instructions:

Each CEL-Seq2 primer is one of 96, each with its own sample barcode. Sequences are in the document "CEL-Seq2 Protocol for Script Hub." These primer stocks should be stored in a 96-well plate at -20 degrees. 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-Seq2 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 (5X)	24.7	1.235	1.0016
dNTP Mix (each) (25 mM)	1	0.25	0.2028
E coli DNA Polymerase I (10 U/µl)	3.3	0.33	0.2676
RNase H (2 U/µl)	1	0.02	0.0162
C1 Loading Reagent (20X)	5	1	0.811
Water	64		
E coli Ligase (10 U/µl)	1	0.1	0.0811
	100 Total Prep Volume		

Protocol Reagent Shopping List

Reagent Name	Vendor	Part Number	Kit Part Number	Stock Concentration
RNaseOUT	Thermo Fisher	10777019		40 U/µl
1:10,000 dilution of ERCC RNA Spike-In Mix	Ambion	4456740		0.01%
NP-40	Thermo Fisher	28324		10%
Tris-HCl, pH 8.4	Teknova	T1084		1000 mM
EDTA	Teknova	E0306		500 mM
DTT (dithiothreitol)	Teknova	D9750		1000
dNTP Mix (each)	Thermo Fisher	R1121		25 mM
SuperScript II Reverse Transcriptase	Thermo Fisher	18064014		200 U/µl
T7 Reaction Buffer	Thermo Fisher		AM1334	10X
T7 Enzyme Mix	Thermo Fisher		AM1334	10X
rNTP Mix (each)	New England BioLabs	N0466S		25 mM
Second Strand Buffer	Thermo Fisher	10812014		5X
E coli DNA Polymerase I	Thermo Fisher	18010025		10 U/µl
RNase H	Thermo Fisher	18021071		2 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
C1 Blocking RGT	100-5316	1X	☑	☑	☑	☑	
C1 Harvest RGT	100-6248	1X	☑	☑		☑	
C1 Preloading RGT	100-5311	1X	☑	☑	☑	☑	
C1 Suspension RGT	100-5315	2.5X	☑	☑	☑	☑	
C1 Cell Wash BUF	100-5314	1X	☑	☑	☑		
C1 Loading Reagent	100-5170	20X	☑	☑			☑
C1 Harvest Reagent	100-7081	1X					