

C1 CAGE: Revealing Gene Regulation at single cell resolution

RIKEN CLST DGT
Research Associate
Tsukasa Kouno

Outline of the talk

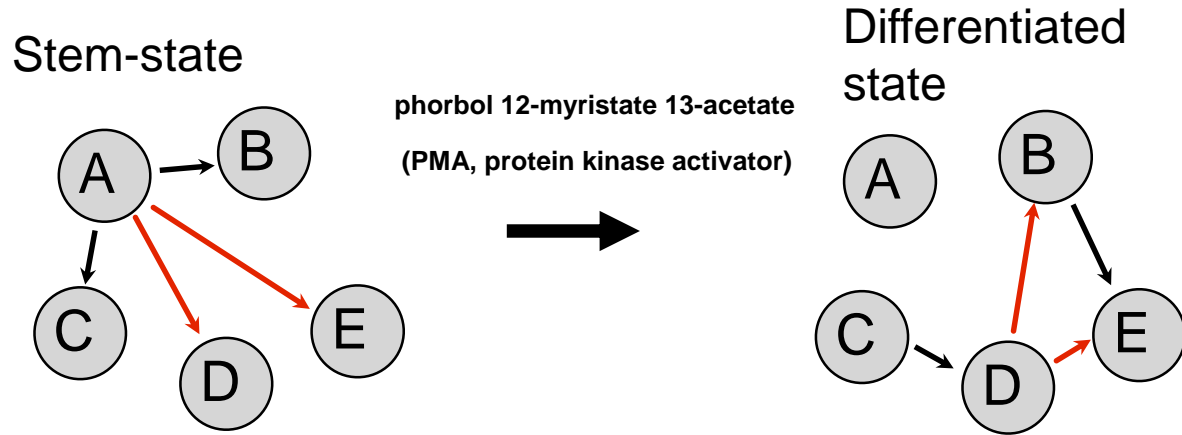
- *Introduction*

 - Gene network analysis by qPCR quantification from single cell

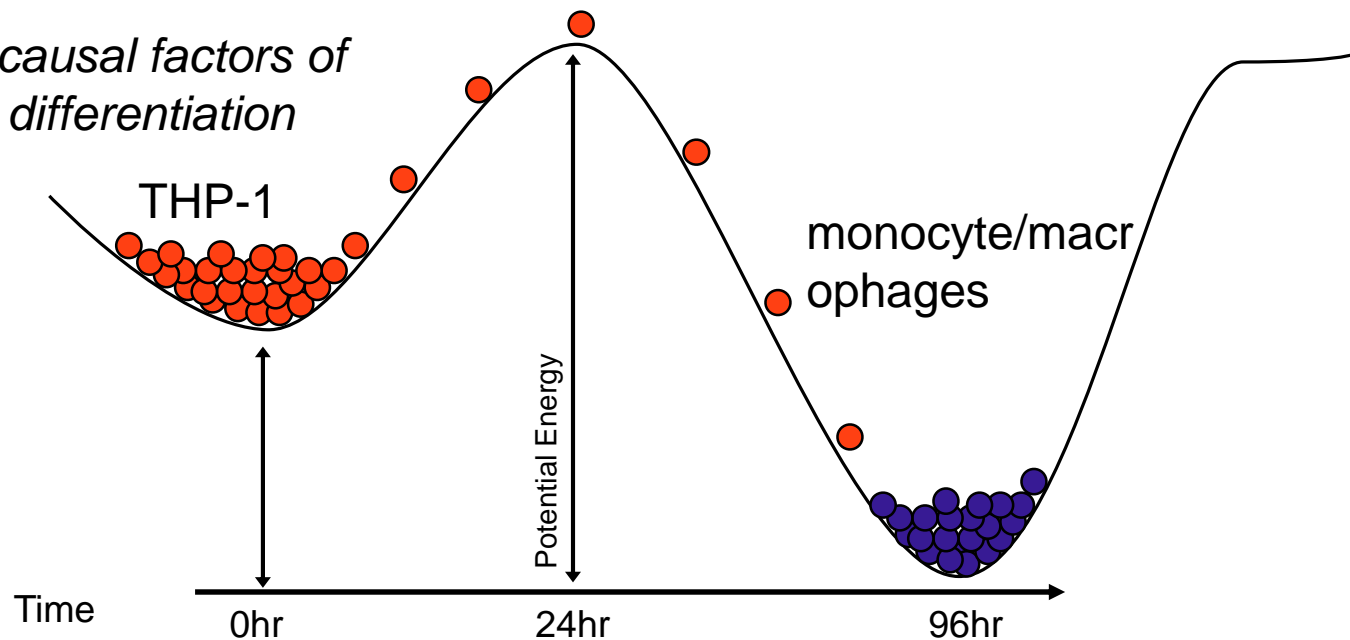
- *Novel method: C1-CAGE*

- *Results and Outlook*

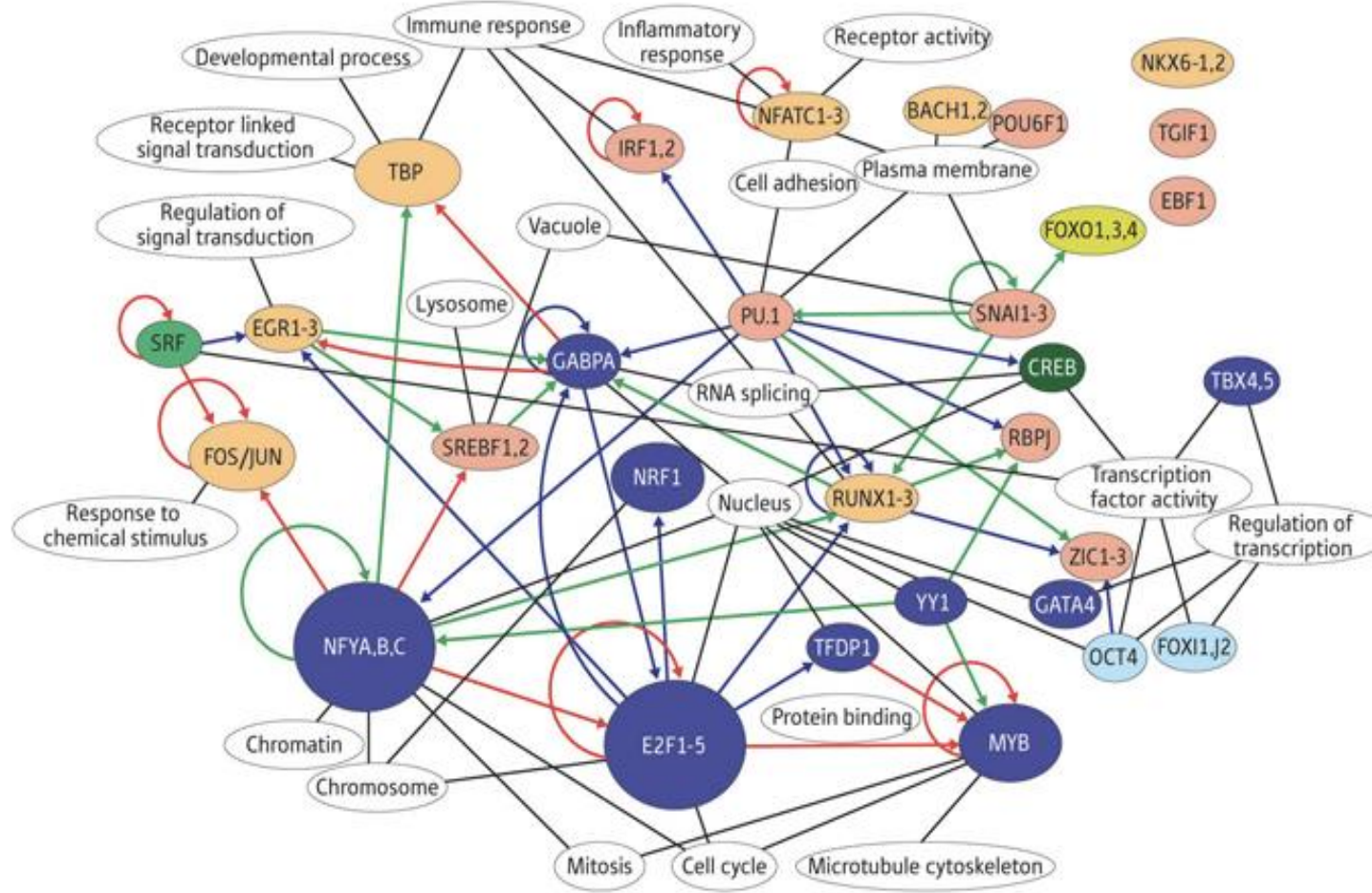
Temporal Dynamics of Transcriptional Regulatory Network (TRN)



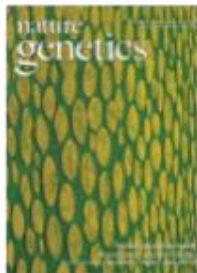
Identify causal factors of myeloid differentiation



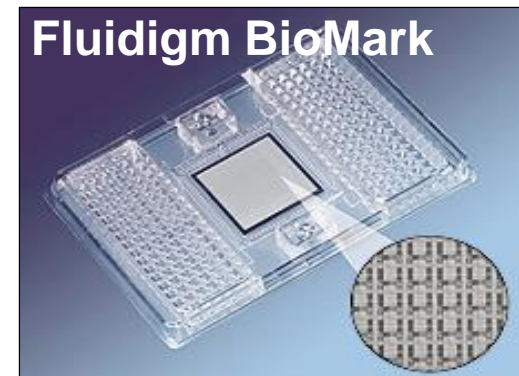
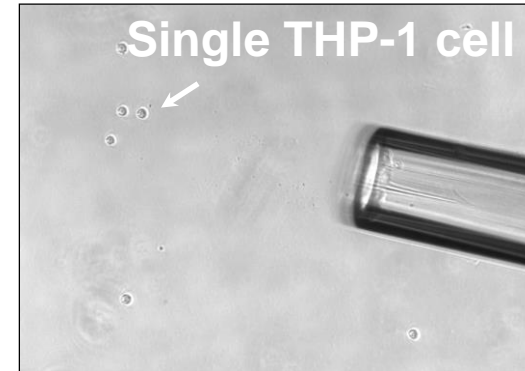
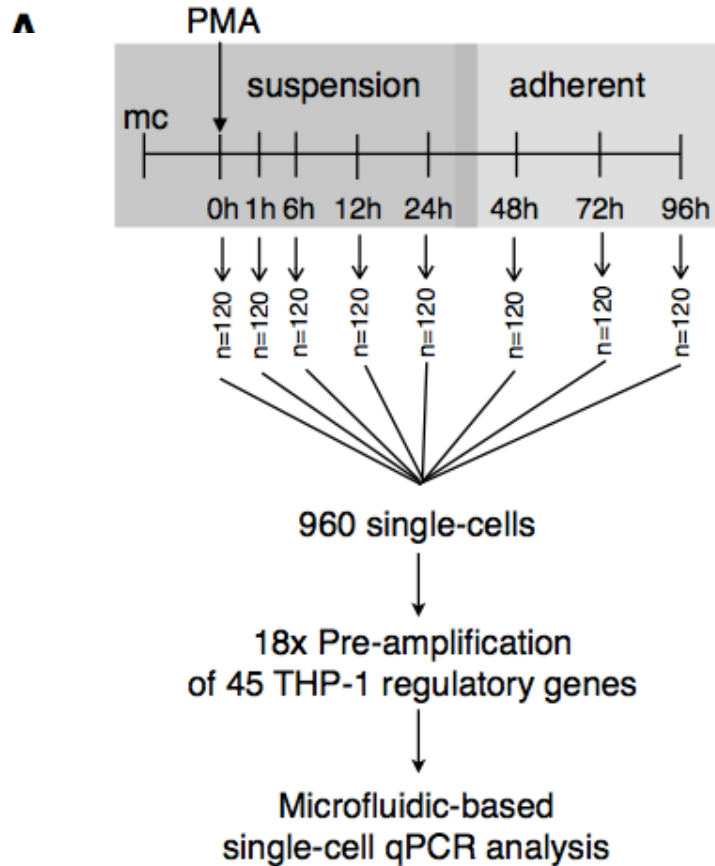
Transcriptional Regulatory Network (TRN) of monocyte differentiation



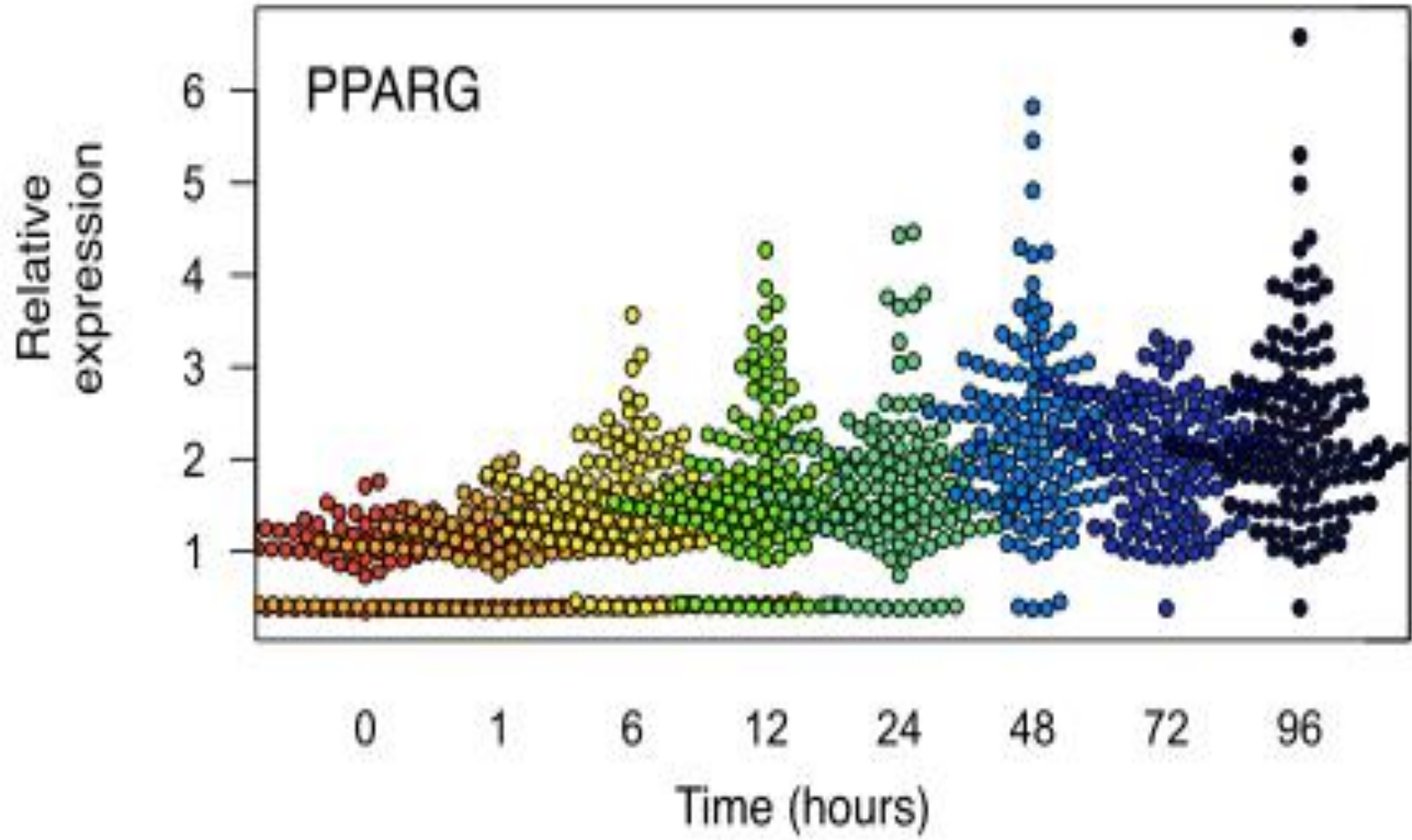
2009



Profiling of PMA-induced THP1 single cells

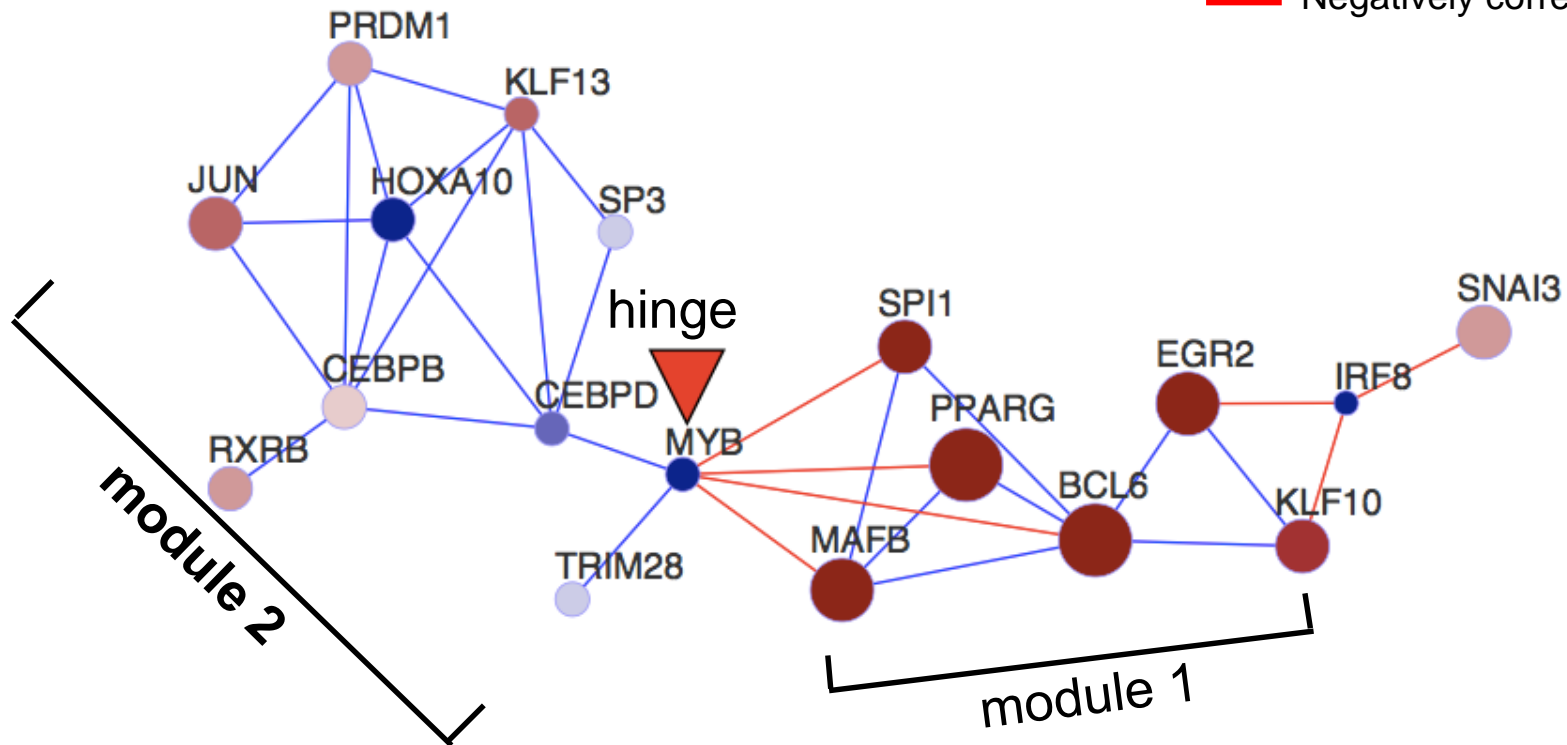


PPARG expression at single cell level



MYB acts as a network-hinge controlling positive and negative co-expression networks

— Positively correlated
— Negatively correlated



*Comprehensive gene detection
at the single cell level...*



Fluidigm C1 system

Single-cell RNA-seq (C1 RNAseq)

Poly-dT primed, Tagmentation,
Exon coverage

Single-cell CAGE (C1 CAGE)

Random primed, Tagmentation
TSS specific

Workflow

Cell preparation

Loading/
capture

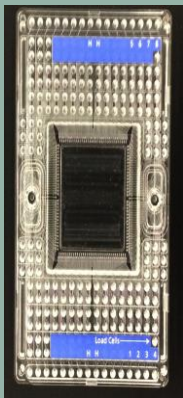
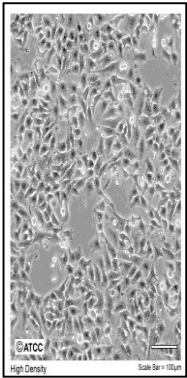
Imaging/
Fluorescence
measurement

Lysis, RT,
Amplification

Library
preparation

Sequencing

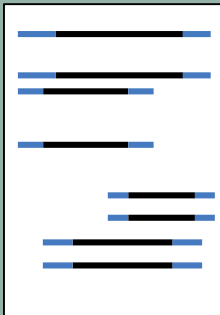
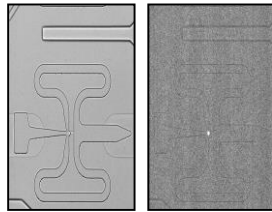
Analysis



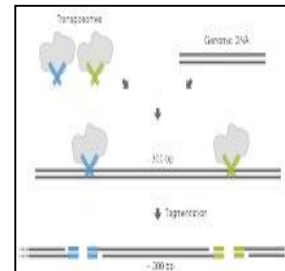
C1 IFCs



INCellAnalyzer 6000



Clontech
SMARTer
Ultra Low
RNA Kit



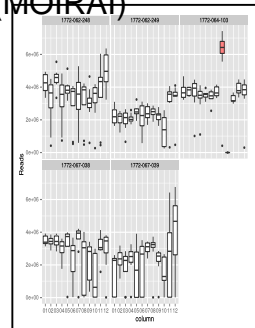
Nextera
XT DNA Sample
Preparation/
Index Kit



Read files and
processing
(MOIRAI)



Illumina HiSeq2500 sequencer



GitHub
command line



C1 Single-Cell Auto Prep System

Single cell database

SINGLE-CELL DATA INTEGRATION PLATFORM

"A resource for the single-cell research community"

HeLa Fucci cells » Fucci Metadata » A549 cells » Non-RIKEN single-cell datasets » A user guide » Home page

With selected... More... Search for: search

Details found: 480 [1 2 3 4 5 6 7 8 9 10] Next : Last Page 1 of 40 Records Per Page: 12

Cell id	cDNA concentration	Error	Fluo QC	Ch2 corrected	Ch3 corrected	Low complexity	SPIKE 1	SPIKE 4	SPIKE 7	SPIKE 3	SPIKE 6	rRNA 18S	rRNA 28S	rRNA 5.8S	Nextera	HPV	HPV As	Control	Reads	HiSeq QC	Discard
Sequencing (1) Cell Images (1) 1772-067-039_H12	0.99	0	FALSE	16.05	55.14	4,094.97	107.38	0.86	0.00	0.00	0.00	490.41	62.31	1.44	95,808.50	1,345.75	504.76	NA	3482812	TRUE	FALSE
Sequencing (1) Cell Images (1) 1772-067-039_H11	0.76	0	FALSE	12.93	18.34	4,089.20	158.96	18.01	0.00	0.00	0.00	415.41	42.22	0.93	99,136.09	1,171.40	434.97	NA	3220926	TRUE	FALSE
Sequencing (1) Cell Images (1) 1772-067-039_H10	0.20	0	FALSE	12.68	12.87	11,482.60	1,870.26	2.01	22.09	0.00	0.00	447.74	84.33	8.03	129,280.12	31.12	23.09	NA	996116	TRUE	FALSE
Sequencing (1) Cell Images (1) 1772-067-039_H09	1.88	0	FALSE	78.01	14.84	4,272.88	121.15	1.11	0.00	0.00	0.00	695.41	57.43	6.30	92,003.76	2,015.10	729.50	NA	2699118	TRUE	FALSE
Sequencing (1) Cell Images (1) 1772-067-039_H08	1.13	0	FALSE	17.85	85.97	4,167.22	141.74	0.63	1.26	0.00	0.00	535.78	73.71	4.09	88,573.64	1,412.70	511.85	NA	3174782	TRUE	FALSE

Proceed to Cell Images

Details found: 1 Page 1 of 1

Cell id	Bright field	Green fluorescence	Red fluorescence
1772-067-039_H12			

Sequencing (1) Cell Images (1) 1772-067-039_H11

Sequencing (1) Cell Images (1) 1772-067-039_H10

Proceed to Sequencing

Details found: 1 Page 1 of 1

Cell id	DDBJ run accession number	Barcode	Library	Experiment accession	Well	Run				
Genomic coordinates and expression (1) FASTQ ChId Table (2) 1772-062-248_A01	DRR028211	TAAGGCGA-TAGATCGC	RNh10371	DRX019711	A01	1772-062-248				
Sequencing (1) Cell Images (1) 1772-062-248_A02	0.17	1	TRUE	0.00	0.00	34,459.27	5,752.50	182.57	19.68	0.00
Sequencing (1) Cell Images (1) 1772-062-248_A03	2.83	0	FALSE	14.08	30.26	2,430.86	6,193.90	657.04	20.22	0.00

Cell ID
cDNA concentration
Fluorescence intensity
Read number
etc.

← Cell Images

← Sequencing



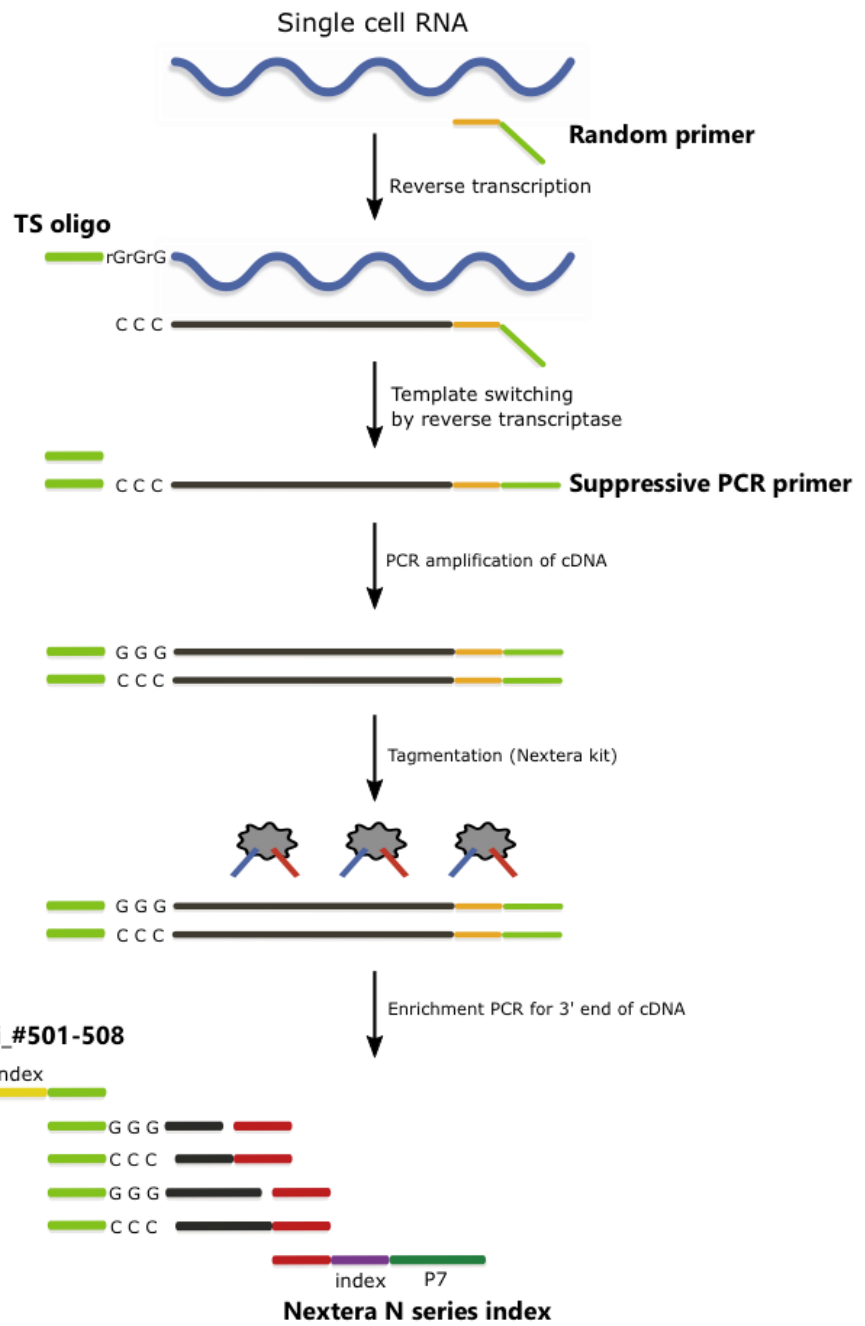
C1 system

Single-cell RNA-seq (C1 RNAseq)

Poly-dT primed, Tagmentation,
exon coverage

Single-cell CAGE (C1 CAGE)

Random primed, Tagmentation
TSS specific (5' ends)

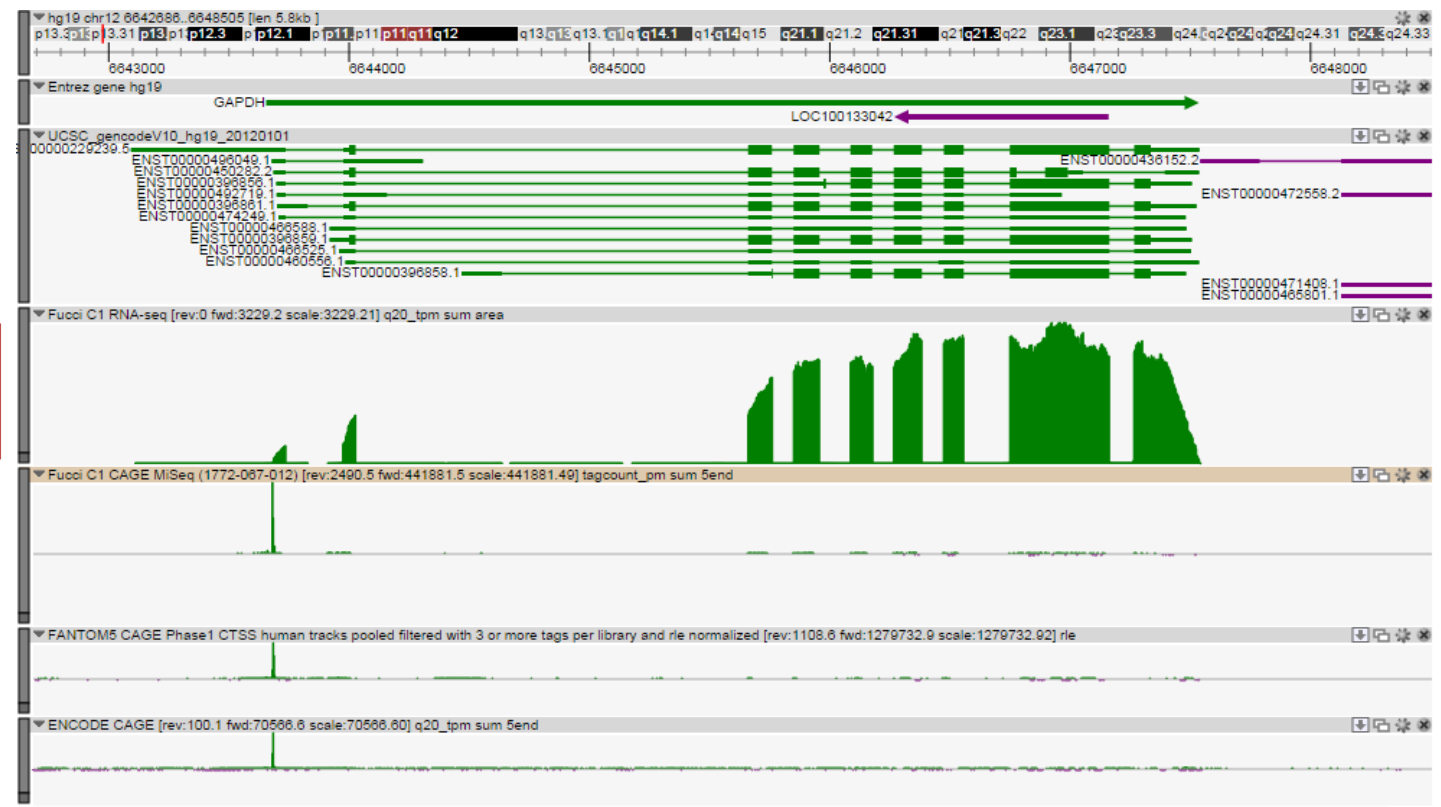


1

2

GAPDH

C1 RNAseq
C1 CAGE
FANTOM5 CAGE
ENCODE CAGE



Each Single cell

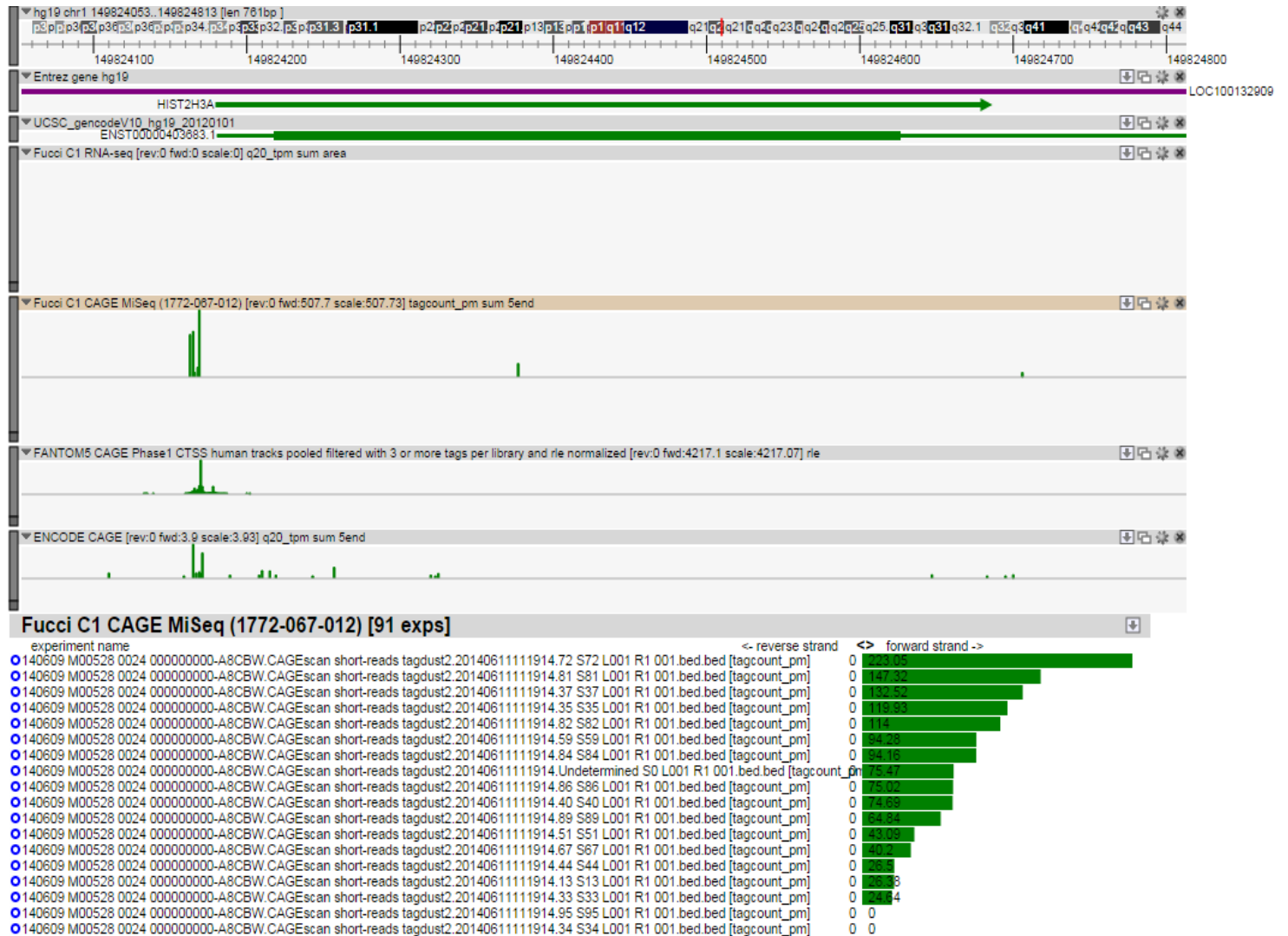
Fucci C1 CAGE MiSeq (1772-067-012) [91 expts]

experiment name	<- reverse strand	<> forward strand >>
140609 M00528 0024 000000000-A8CBW.CAGEscan short-reads tagdust2.20140611111914.18 S18 L001 R1 001.bed.bed [tagcount_pm]	0	1173.13
140609 M00528 0024 000000000-A8CBW.CAGEscan short-reads tagdust2.20140611111914.42 S42 L001 R1 001.bed.bed [tagcount_pm]	0	1363.13
140609 M00528 0024 000000000-A8CBW.CAGEscan short-reads tagdust2.20140611111914.91 S91 L001 R1 001.bed.bed [tagcount_pm]	73.65	1343.04
140609 M00528 0024 000000000-A8CBW.CAGEscan short-reads tagdust2.20140611111914.24 S24 L001 R1 001.bed.bed [tagcount_pm]	0	1313.47
140609 M00528 0024 000000000-A8CBW.CAGEscan short-reads tagdust2.20140611111914.66 S66 L001 R1 001.bed.bed [tagcount_pm]	28.95	1233.94
140609 M00528 0024 000000000-A8CBW.CAGEscan short-reads tagdust2.20140611111914.9 S9 L001 R1 001.bed.bed [tagcount_pm]	304.74	1165.95
140609 M00528 0024 000000000-A8CBW.CAGEscan short-reads tagdust2.20140611111914.22 S22 L001 R1 001.bed.bed [tagcount_pm]	70.98	1124.11
140609 M00528 0024 000000000-A8CBW.CAGEscan short-reads tagdust2.20140611111914.59 S59 L001 R1 001.bed.bed [tagcount_pm]	439.96	1052.96
140609 M00528 0024 000000000-A8CBW.CAGEscan short-reads tagdust2.20140611111914.55 S55 L001 R1 001.bed.bed [tagcount_pm]	150.19	1067.67
140609 M00528 0024 000000000-A8CBW.CAGEscan short-reads tagdust2.20140611111914.76 S76 L001 R1 001.bed.bed [tagcount_pm]	0	1030.34
140609 M00528 0024 000000000-A8CBW.CAGEscan short-reads tagdust2.20140611111914.74 S74 L001 R1 001.bed.bed [tagcount_pm]	141.76	1056.33
140609 M00528 0024 000000000-A8CBW.CAGEscan short-reads tagdust2.20140611111914.86 S86 L001 R1 001.bed.bed [tagcount_pm]	150.04	1054.26
140609 M00528 0024 000000000-A8CBW.CAGEscan short-reads tagdust2.20140611111914.8 S8 L001 R1 001.bed.bed [tagcount_pm]	341.2	1034.97
140609 M00528 0024 000000000-A8CBW.CAGEscan short-reads tagdust2.20140611111914.63 S63 L001 R1 001.bed.bed [tagcount_pm]	253.9	1075.48

(TPM)

C1 CAGE enables to read TSS specific for most transcripts. It shows peaks comparable to bulk sample.

HIST2H3A



C1 RNAseq

C1 CAGE

FANTOM5 CAGE

ENCODE CAGE

Each Single cell

C1 CAGE enables sequencing transcripts without polyA.
C1 RNAseq does not.

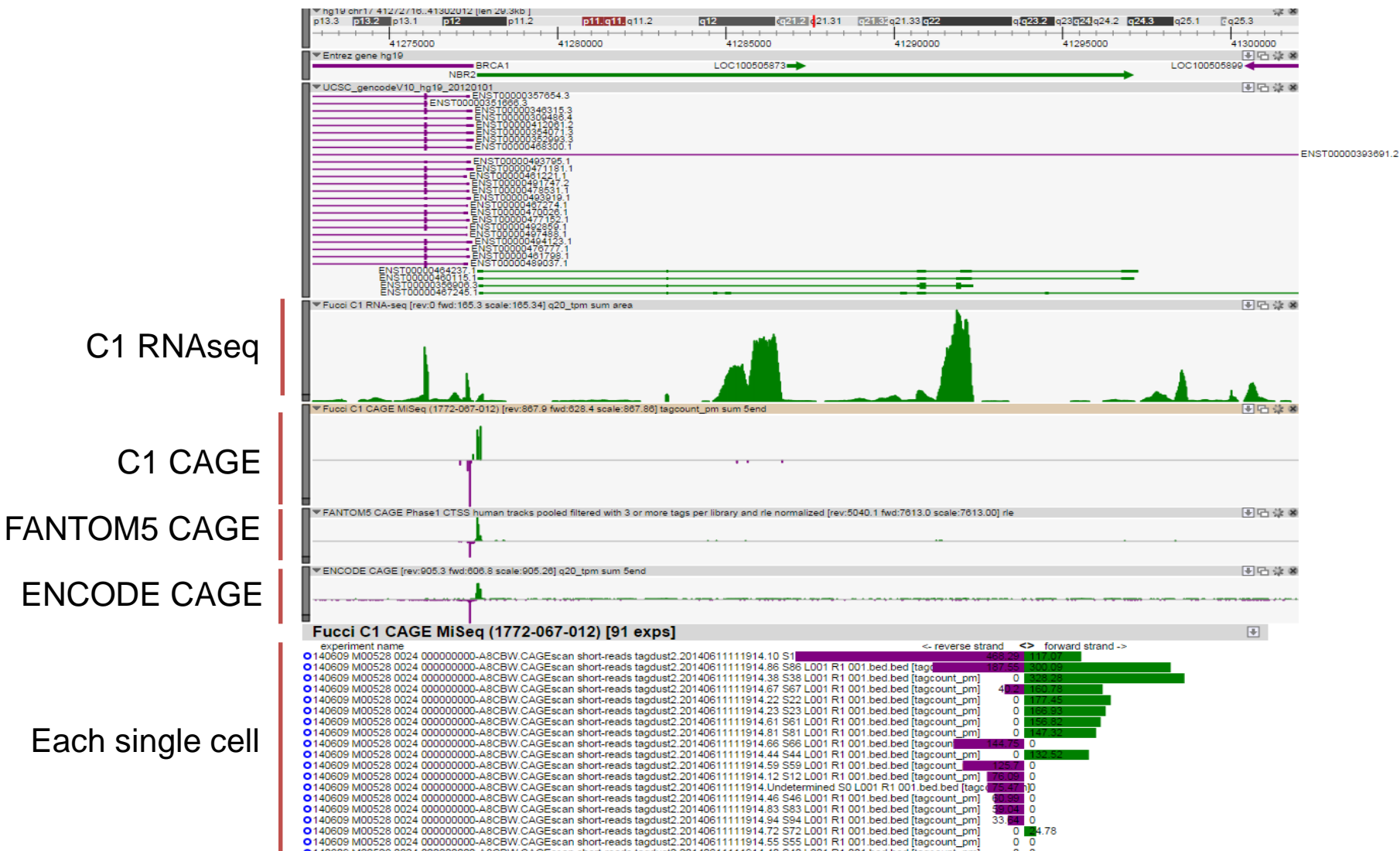
HOTAIR

C1 RNAseq
C1 CAGE
FANTOM5 CAGE
ENCODE CAGE



C1 CAGE enables to read directionality.
Only a few cells reproducibly expressed anti-sense RNA.

Bidirectional



C1 CAGE enables to detect bi-directional promoters.
Each promoter is expressed dominantly.

ScriptHub



C1 CAGE mRNA Sequencing

T. Kouno, S. Kato, M. Mendez, I. Abugessaisa, J. Shin and C. Plessy

Division of Genomics Technologies, Center for Life Sciences Technologies, RIKEN,
JAPAN



VALIDATION



USER RATING

NO REVIEWS YET

[RNA Seq End Counting UMI](#)

PUBLISHED ON 10月 24, 2015 [REV A >](#)

SUPPORTED IFCS: **Open App IFC**

NUMBER OF IFC RUNS: **10**

Overview

C1 CAGE is a method for single-cell transcriptome analysis for molecular counting of RNA 5'-ends. Paired-end sequencing, random priming and unique molecular identifiers are used for single-molecule fragment assembly of mRNAs and long non-coding RNAs, including non-polyadenylated transcripts.

Protocol: C1 CAGE

Duration (H:M): **12:00**

Cell Load



Capture



Stain



Wash

Sample Prep



Lysis



RT



PCR



Harvest

[VIEW PROTOCOL WORKSHEET](#)

(<https://jp.fluidigm.com/c1openapp/scripthub/script/2015-07/c1-cage-1436761405138-3>)

CLST DGT

Yi Huang

Andrew T.Kwon

Efthymios Motakis

Michael Böttcher

Sachi Kato

Imad Abugessaisa

Joachim Luginbühl

Mickaël Mendez

Erik Arner

Piero Carninci

Charles Plessy

Jay W.Shin

Thank you for your attention