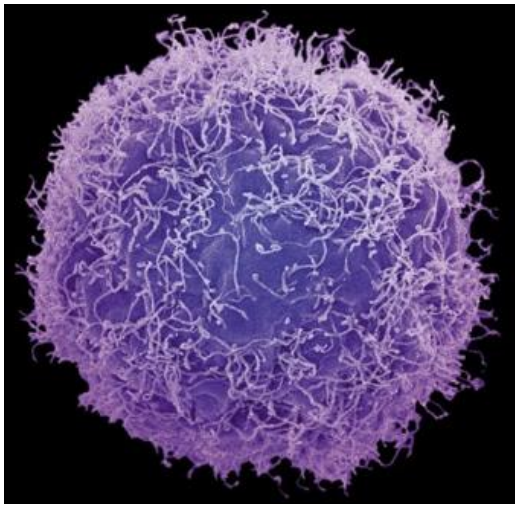


C1 CAGE: Revealing Gene Regulation at single cell resolution

RIKEN CLST DGT

Research Associate

Tsukasa Kouno

Outline of the talk

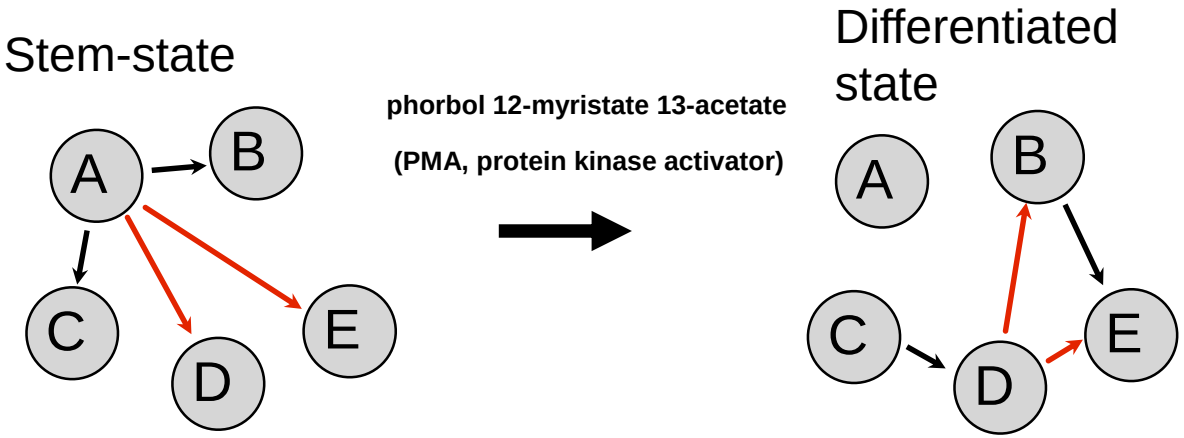
- *Introduction*

  Gene network analysis by qPCR quantification from single cell

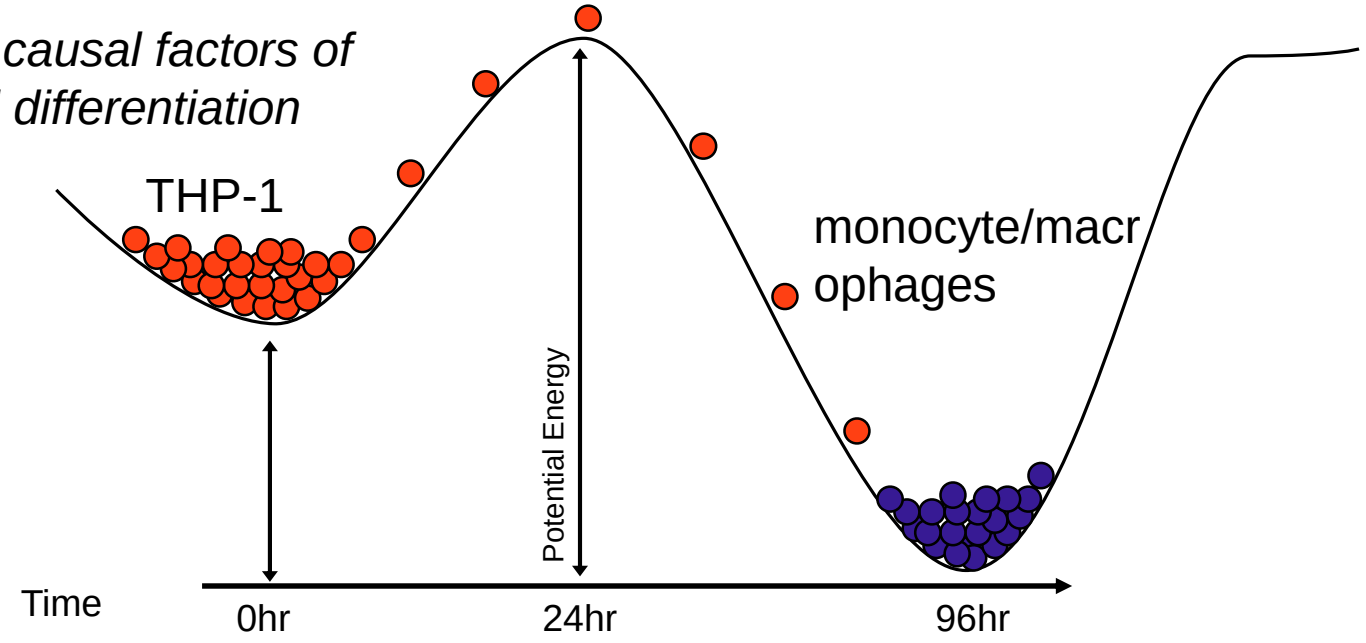
- *Novel method: C1-CAGE*

- *Results and Outlook*

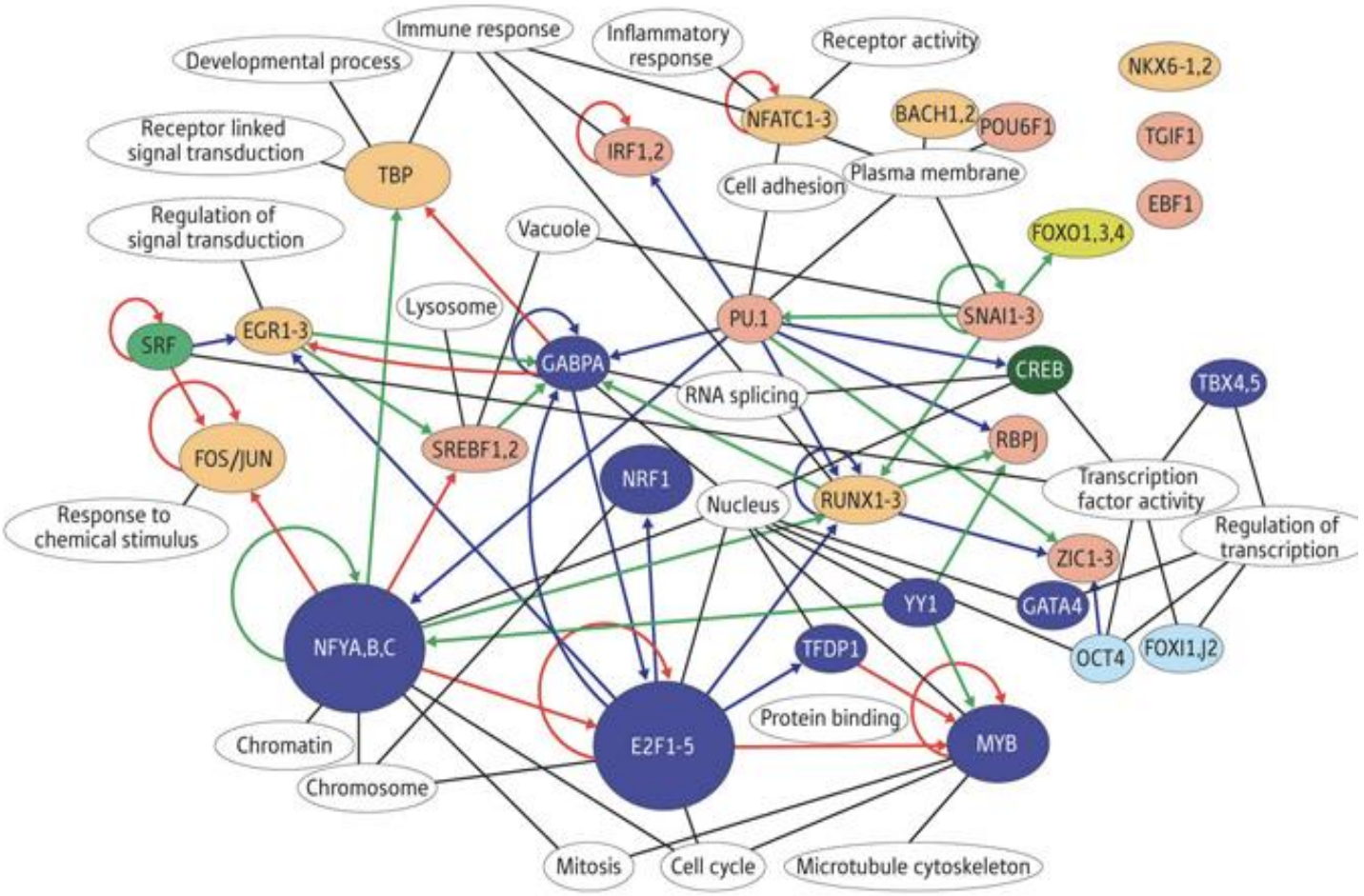
Temporal Dynamics of Transcriptional Regulatory Network (TRN)



Identify causal factors of myeloid differentiation



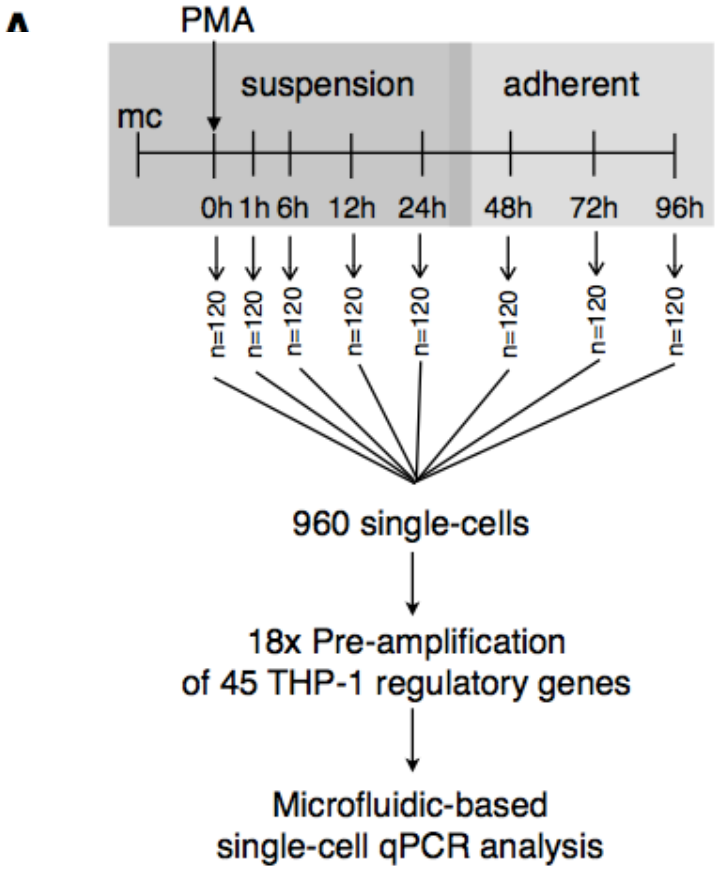
Transcriptional Regulatory Network (TRN) of monocyte differentiation

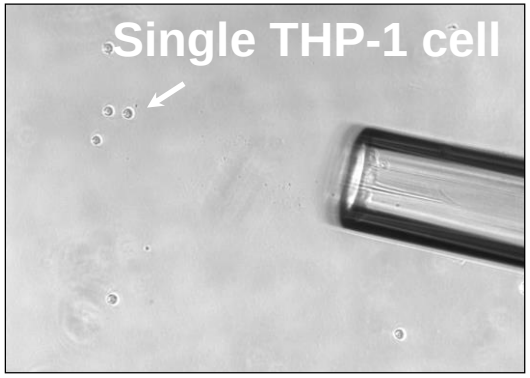


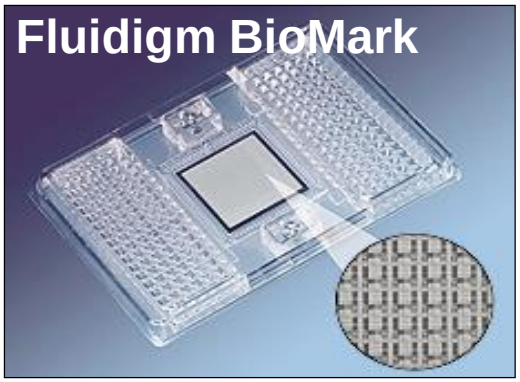
2009

Suzuki H., et al. *Nature Genetics* 2009

Profiling of PMA-induced THP1 single cells

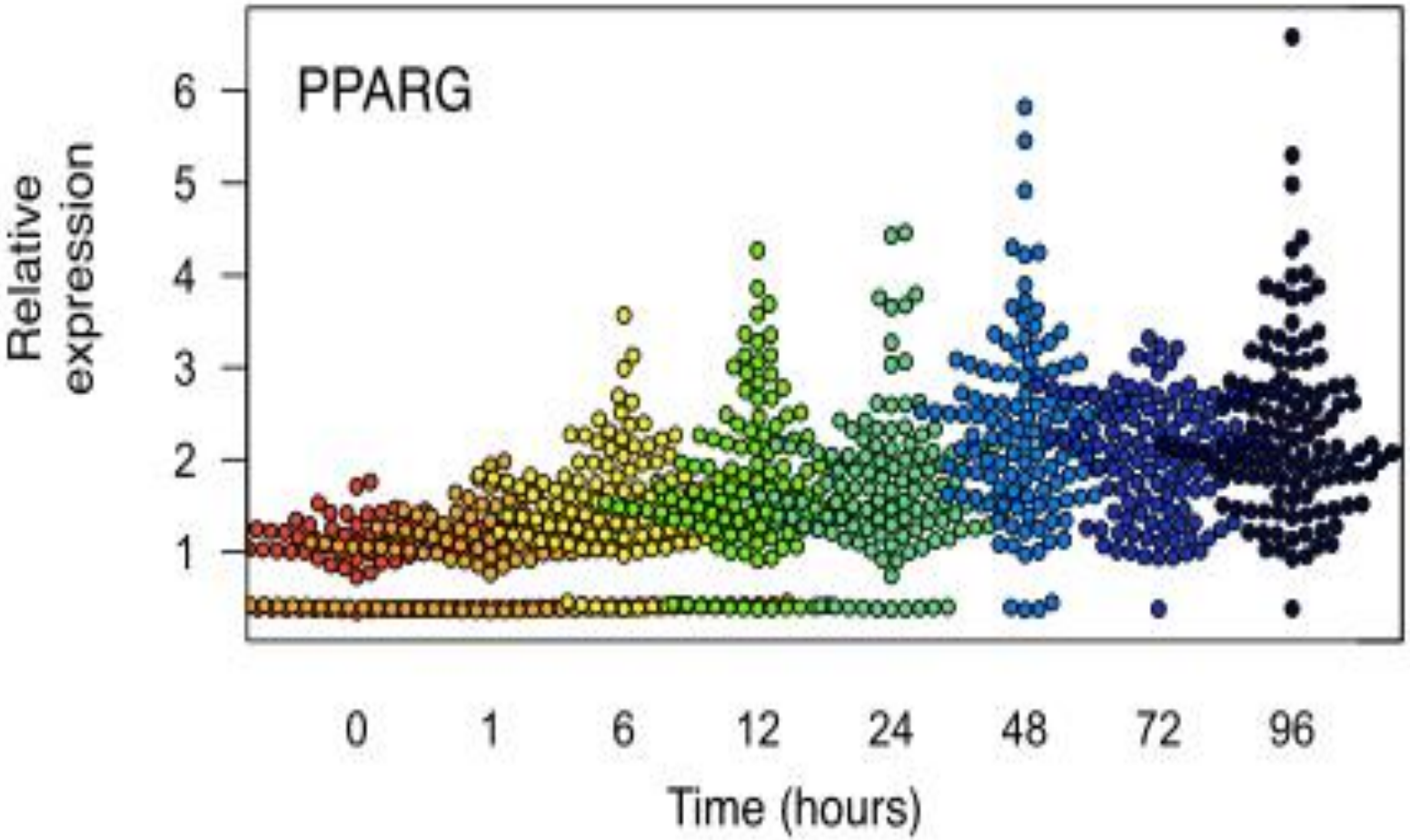






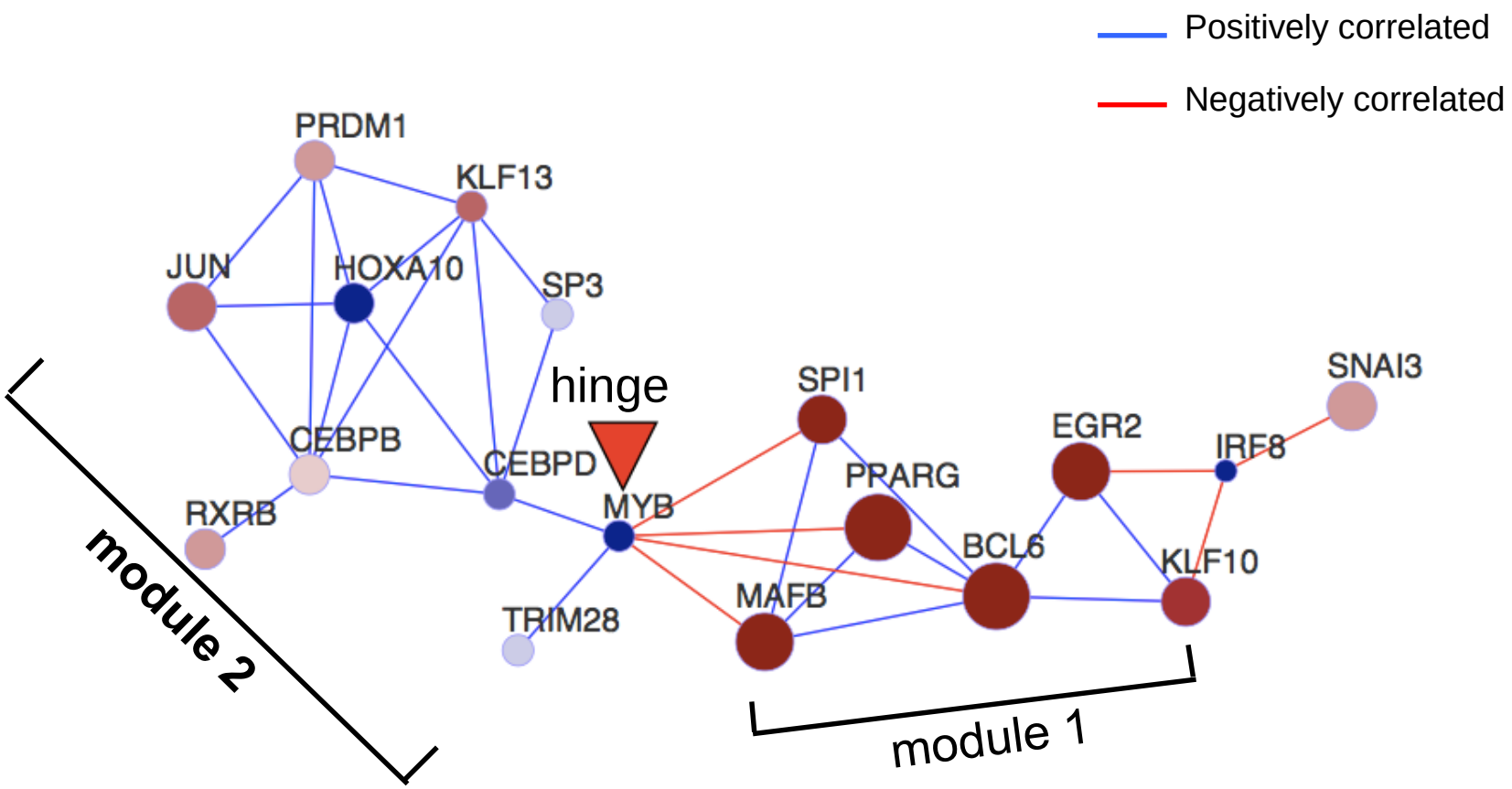
Kouno T., et al. *Genome Biology* 2013

PPARG expression at single cell level



Kouno T., et al. ***Genome Biology*** **2013**

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



Kouno T., et al. *Genome Biology* 2013

Comprehensive gene detection
at the single cell level…

Fluidigm C1 system

Single-cell RNA-seq (C1 RNAseq)

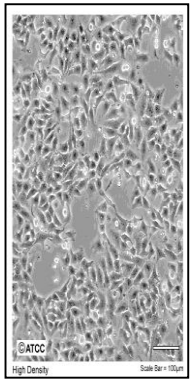
Poly-dT primed, Tagmentation,
Exon coverage

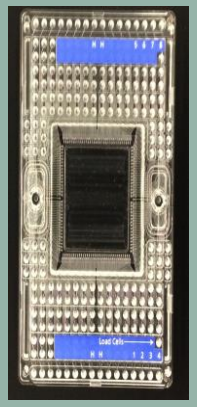
Single-cell CAGE (C1 CAGE)

Random primed, Tagmentation
TSS specific

Workflow

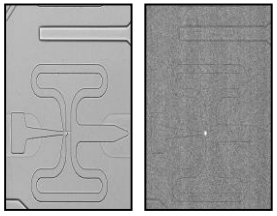
Cell prepararation | Loading/ capture | Imaging/ Fluorescence measurement | Lysis, RT, Amplification | Library preparation | Sequencing | Analysis



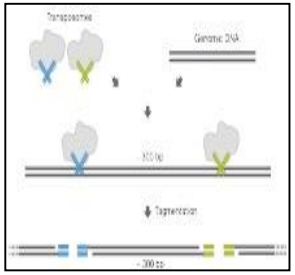


C1 IFCs

INCellAnalyzer 6000



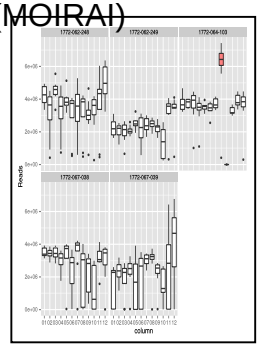
Clontech SMARTer Ultra Low RNA Kit



Nextera XT DNA Sample Preparation/ Index Kit

Illumina HiSeq2500 sequencer

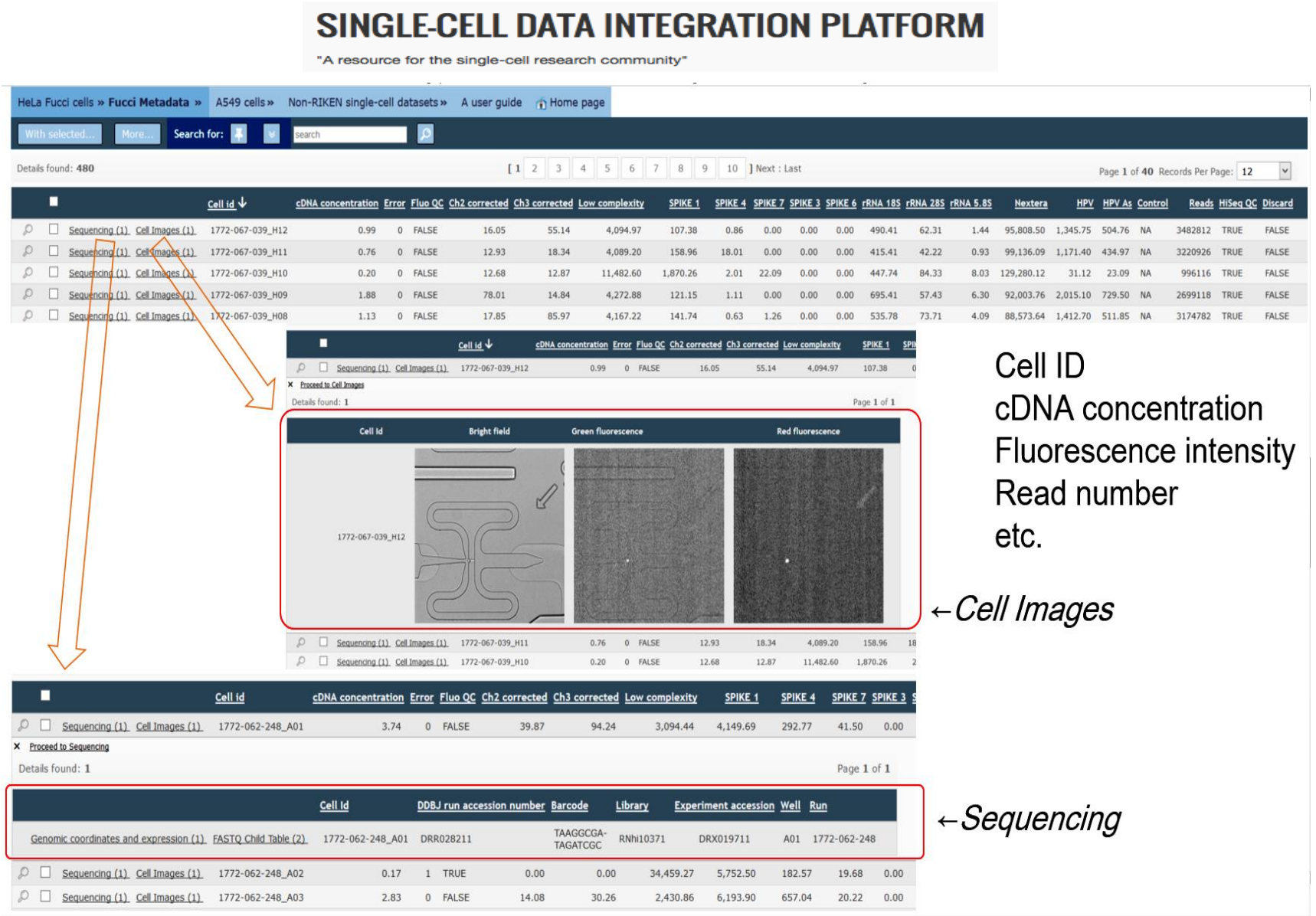
Read files and processing (MOIRAI)



GitHub command line

C1 Single-Cell Auto Prep System

Single cell database



Cell ID
cDNA concentration
Fluorescence intensity
Read number
etc.

←*Cell Images*

←*Sequencing*

http://single-cell.clst.riken.jp

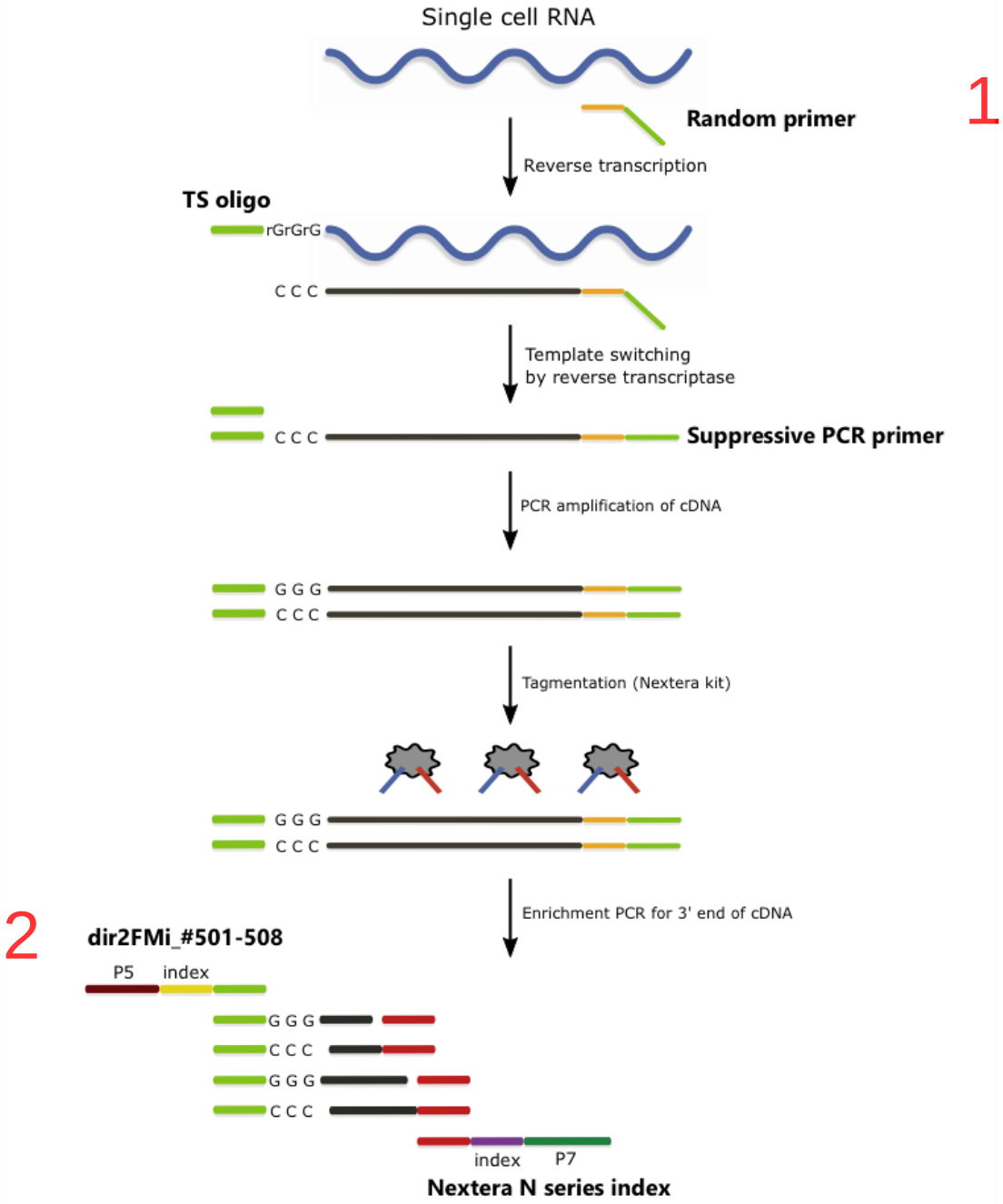
C1 system

Single-cell RNA-seq (C1 RNAseq)

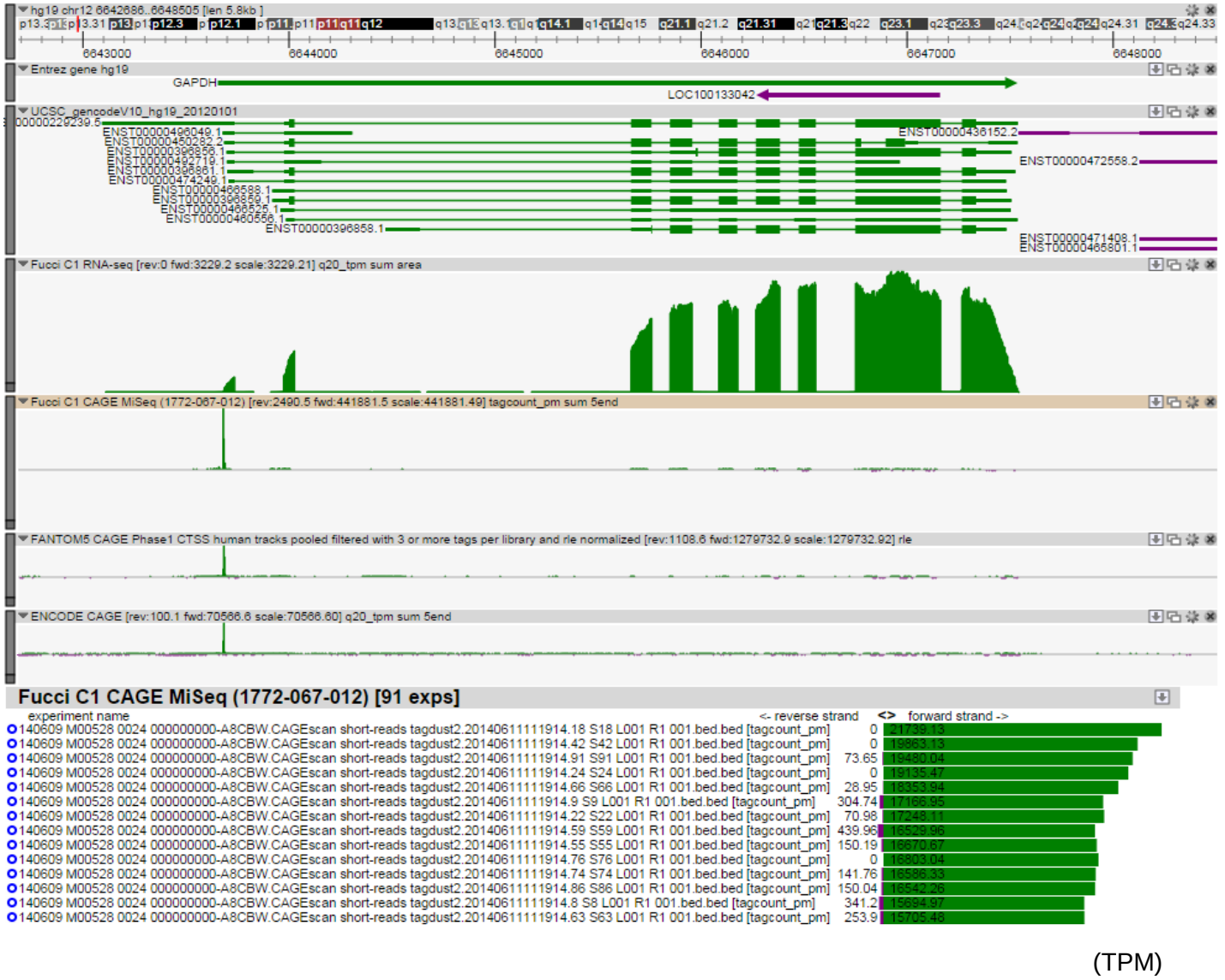
Poly-dT primed, Tagmentation, exon coverage

Single-cell CAGE (C1 CAGE)

Random primed, Tagmentation
TSS specific (5’ ends)


Single cell RNA
Random primer
1
Reverse transcription
TS oligo
rGrGrG
C C C
Template switching
by reverse transcriptase
C C C
Suppressive PCR primer
PCR amplification of cDNA
G G G
C C C
Tagmentation (Nextera kit)
G G G
C C C
Enrichment PCR for 3' end of cDNA
2
dir2FMi_#501-508
P5
index
G G G
C C C
G G G
C C C
index
P7
Nextera N series index

GAPDH



C1 RNAseq

C1 CAGE

FANTOM5 CAGE

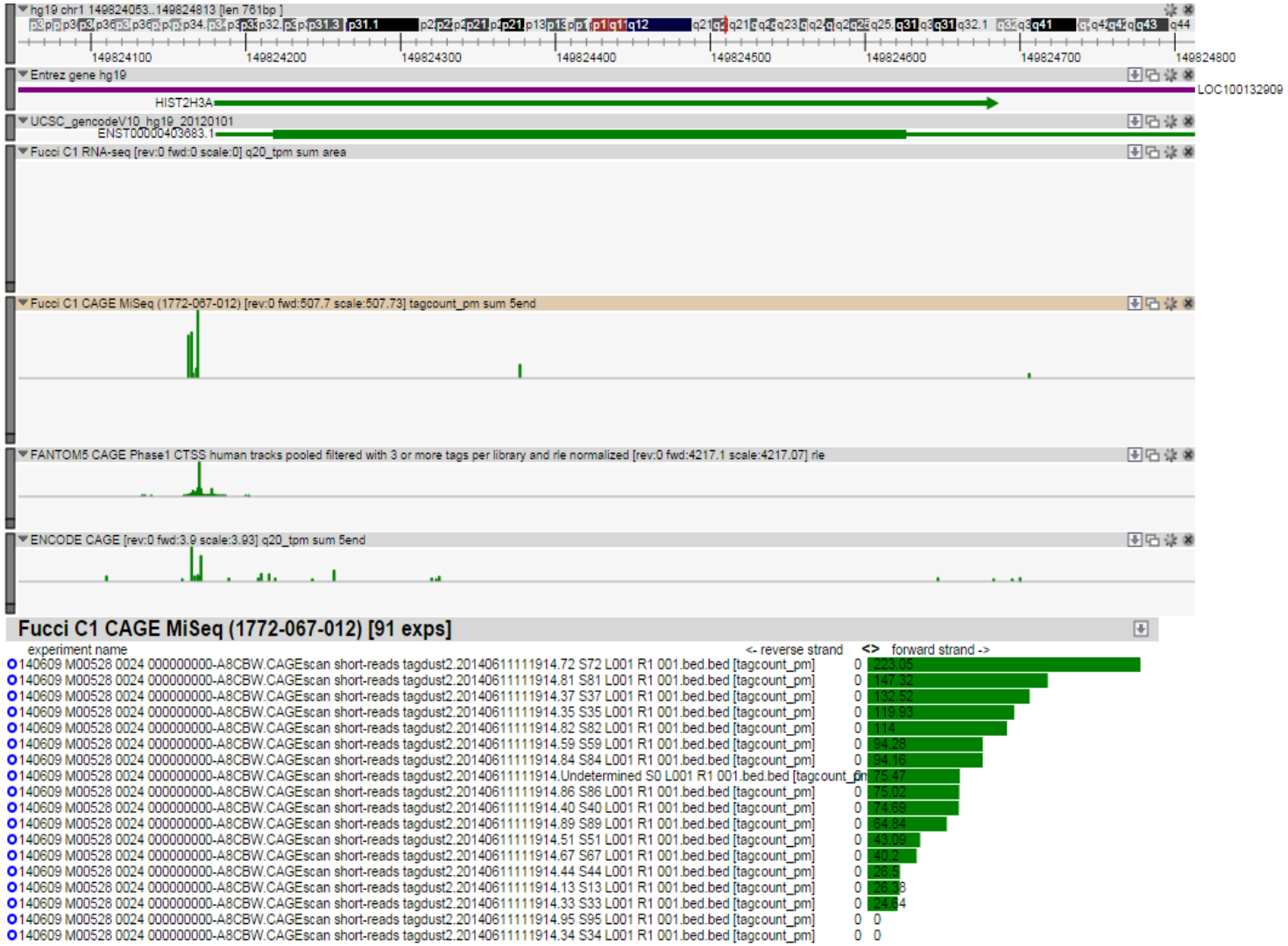
ENCODE CAGE

Each Single cell

(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

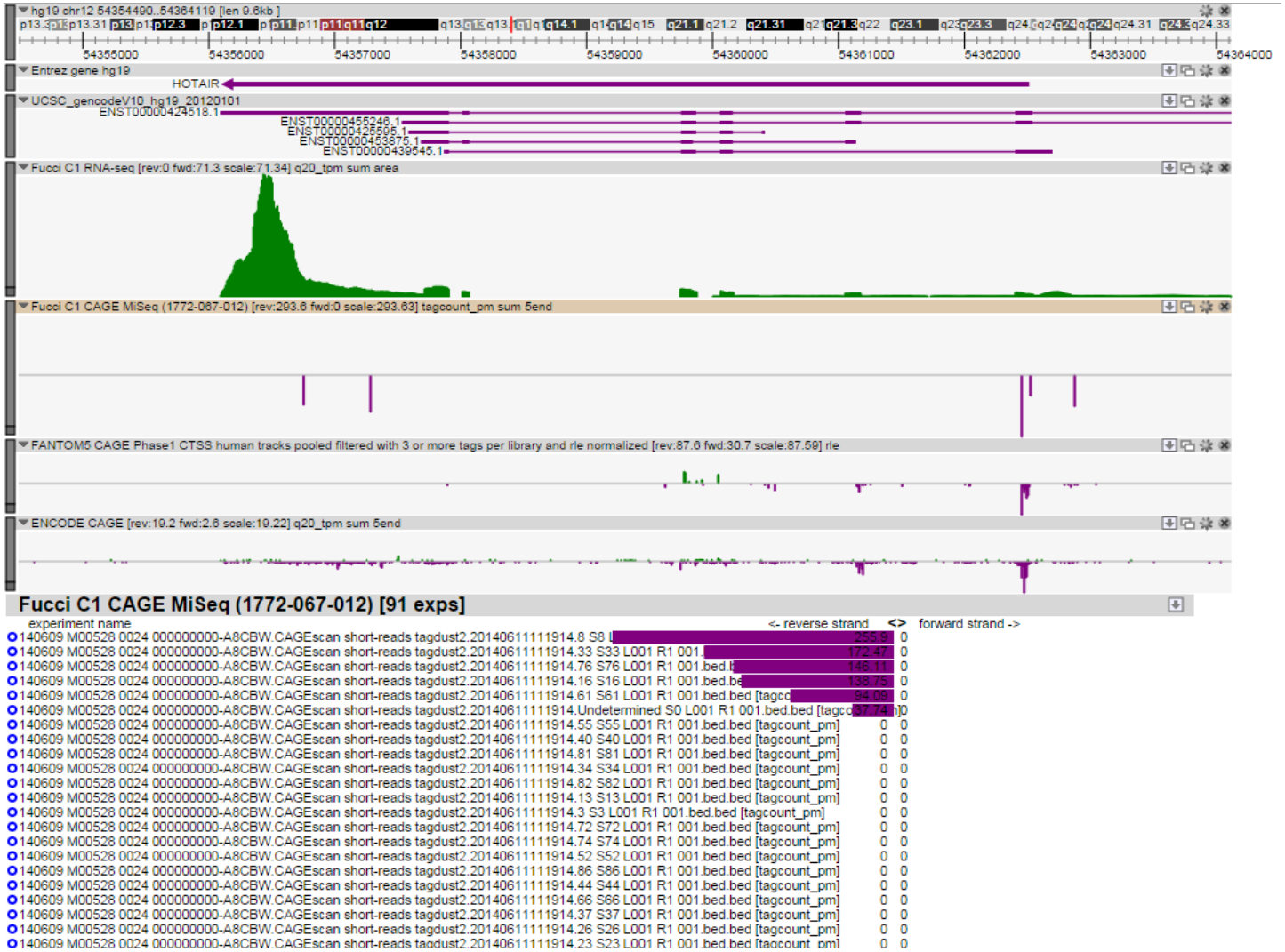
C1 RNAseq

C1 CAGE

FANTOM5 CAGE

ENCODE CAGE

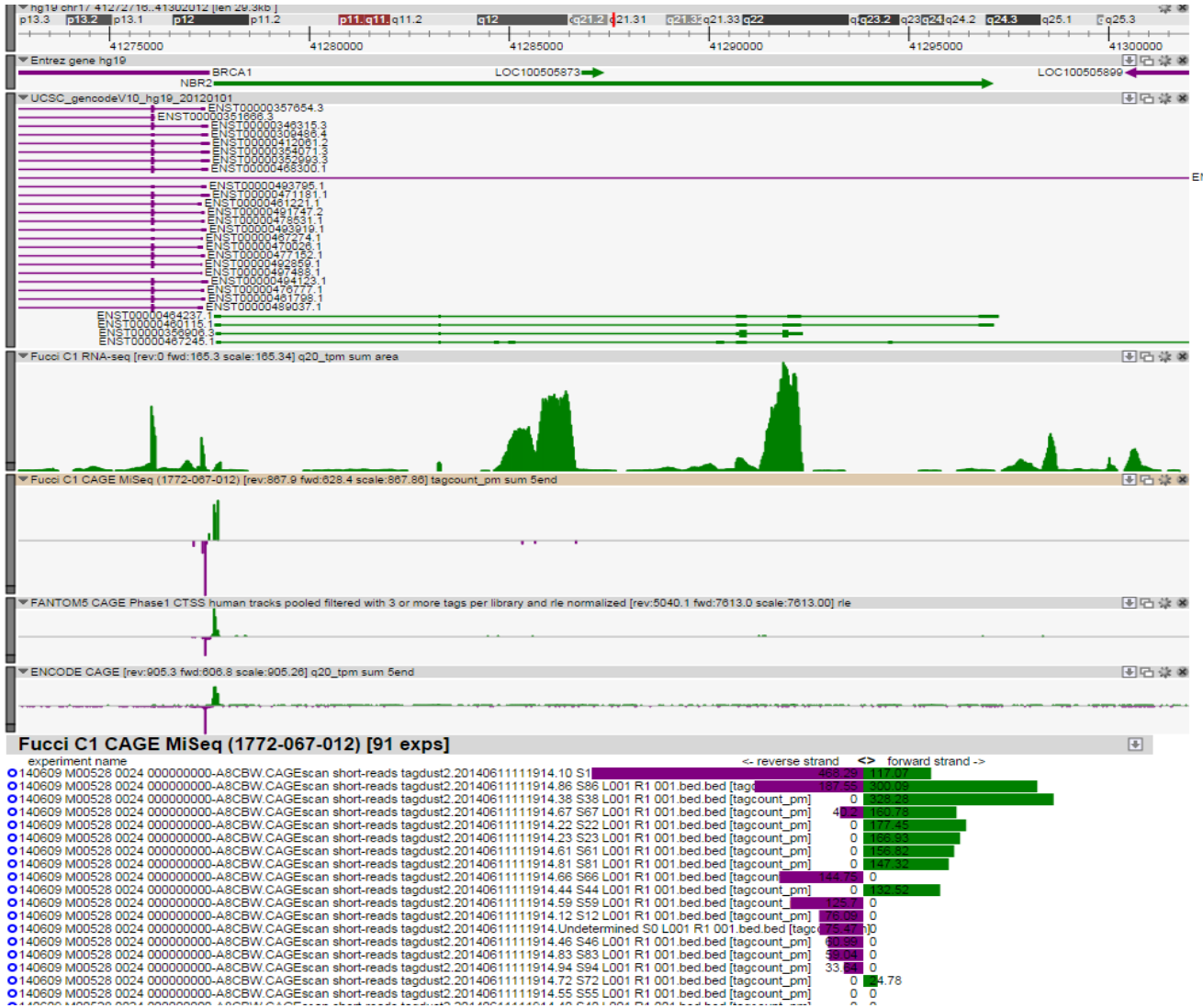
Each Single cell



C1 CAGE enables to read directionality.

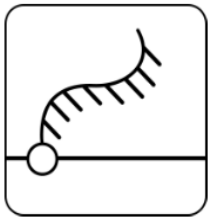
Only a few cells reproduceably expressed anti-sense RNA.

Bidirectional



C1 CAGE enables to detect bi-directiomal 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**

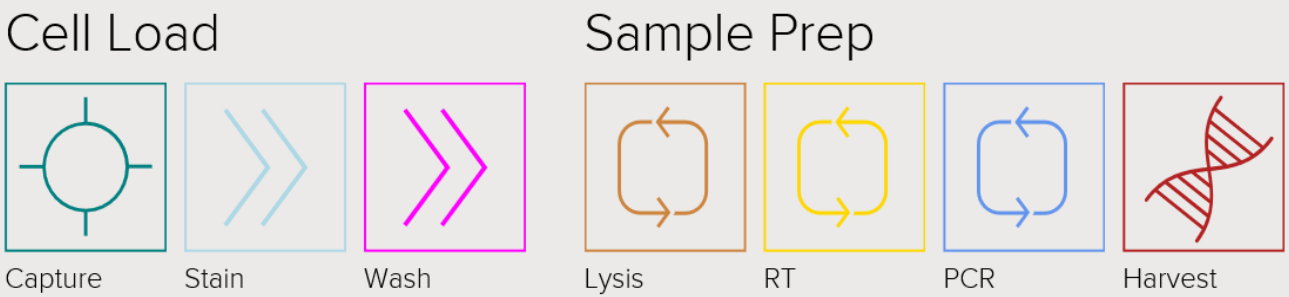
NUMBER OF IFC RUNS: **10**

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**



VIEW PROTOCOL WORKSHEET

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

Acknowledgements

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