2013.2.26

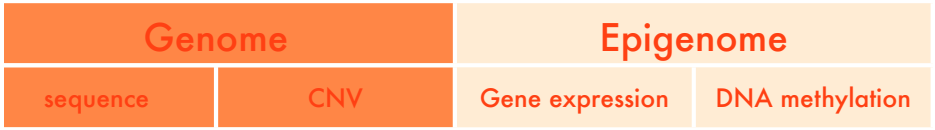
# エピゲノム解析による iPS細胞の特性解析

Center for iPS Cell Research and Application, Kyoto University
Genomics and Epigenomics Core Facility / Yamanaka Lab.
Akira Watanabe

# Genomics and Epigenomics on iPS cell research

Safety

Quality




# 再生医療時代の
# iPS細胞のゲノム解析

# Genomics and Epigenomics on iPS cell research

Safety

Quality

# iPS細胞のクオリティを評価するスタンダードがない！

# 安全なiPS細胞を選べば

# 問題ない！

# 従来の薬

化合物：合成・天然物

HPLC等で精製や純度決定

# 従来の薬

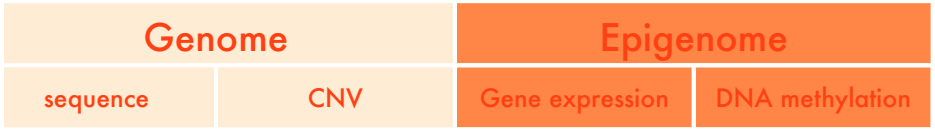
化合物：合成・天然物

HPLC等で精製や純度決定

# 次世代の薬

生細胞

ゲノム・エピゲノム状態の安全性




エピゲノム解析による
iPS細胞の特性解析

Genomics and Epigenomics
on iPS cell research

Safety

Quality

# エピジェネティクスとは？

# 古典的セントラルドグマ

ゲノム
（DNA）

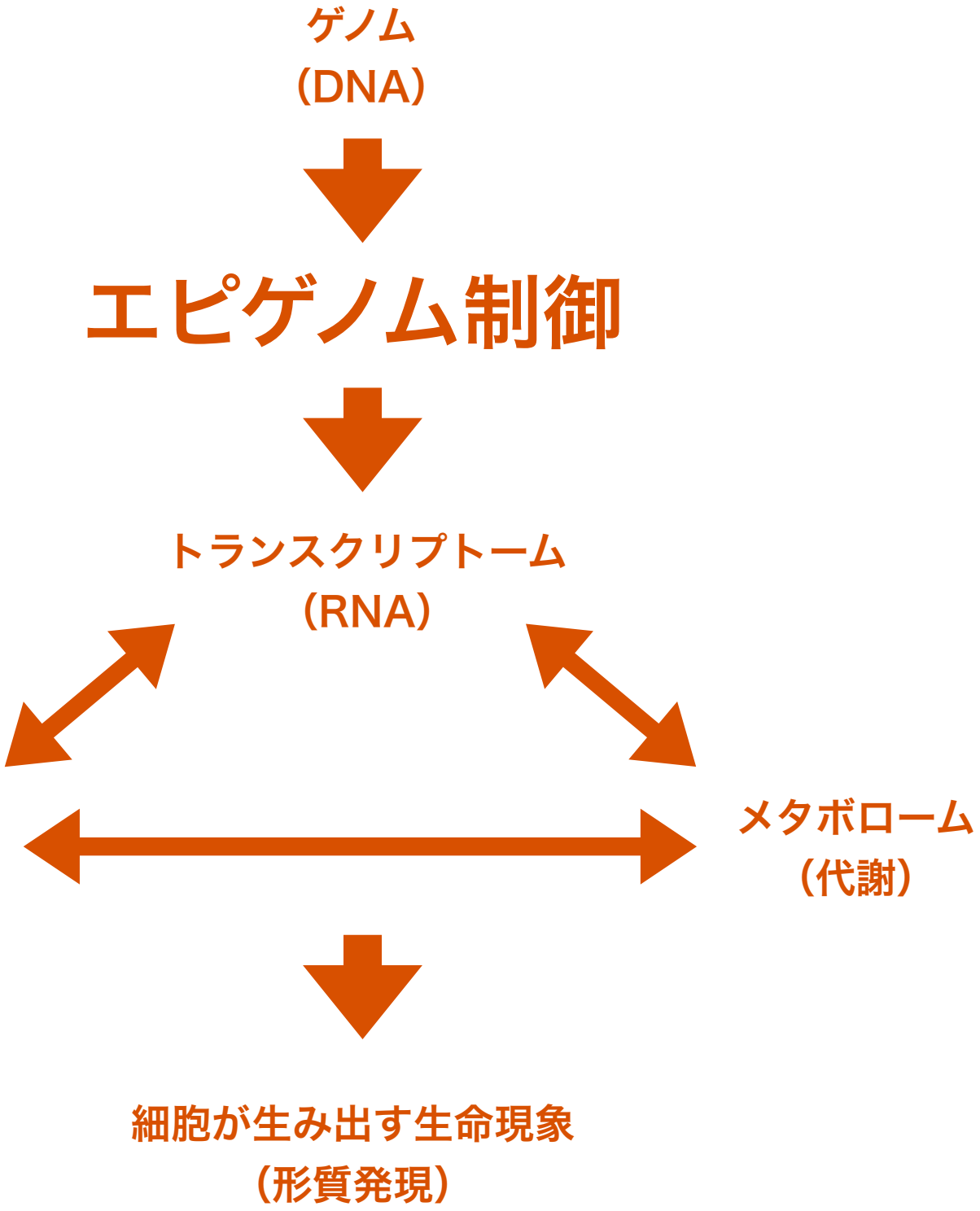
トランスクリプトーム
（RNA）

プロテオーム
（タンパク質）

細胞が生み出す生命現象
（形質発現）

# エピゲノムドグマ


DNA
RNA
タンパク質
形質発現

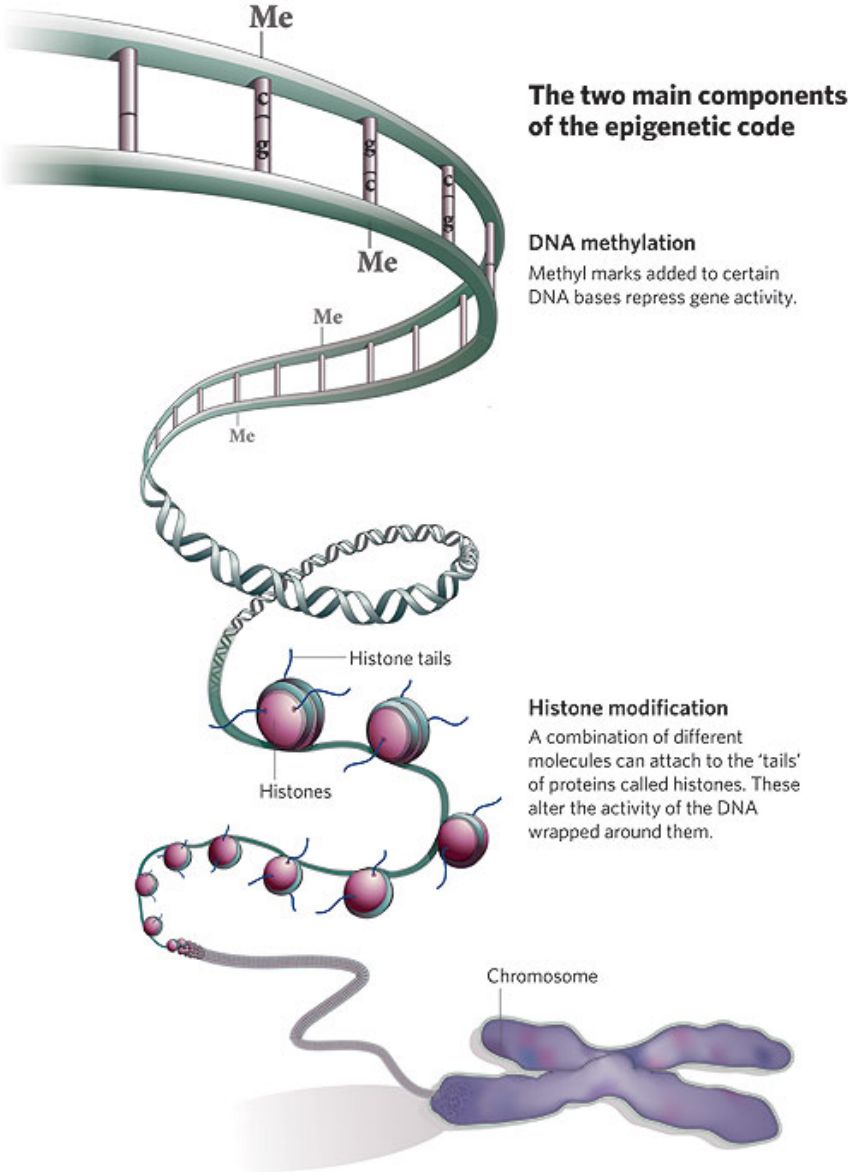

ゲノム
(DNA)
エピゲノム制御
トランスクリプトーム
(RNA)
プロテオーム
(タンパク質)
メタボローム
(代謝)
細胞が生み出す生命現象
(形質発現)

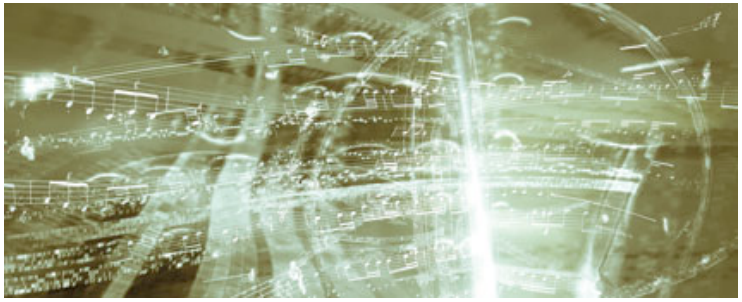
エピジェネティクス（epigenetics）とは、クロマチンへの**後天的な修飾**により遺伝子発現が制御されることに起因する遺伝学あるいは分子生物学の研究分野である。

# エピジェネティクス

# 遺伝子発現制御機構

# エピジェネティックコード



## 転写研究における未完成交響曲

(Qui, Nature 2006)

# ヒストン修飾

# DNAメチル化

# ヒストン修飾は遺伝子発現状態のマークである

H3K4me1, active

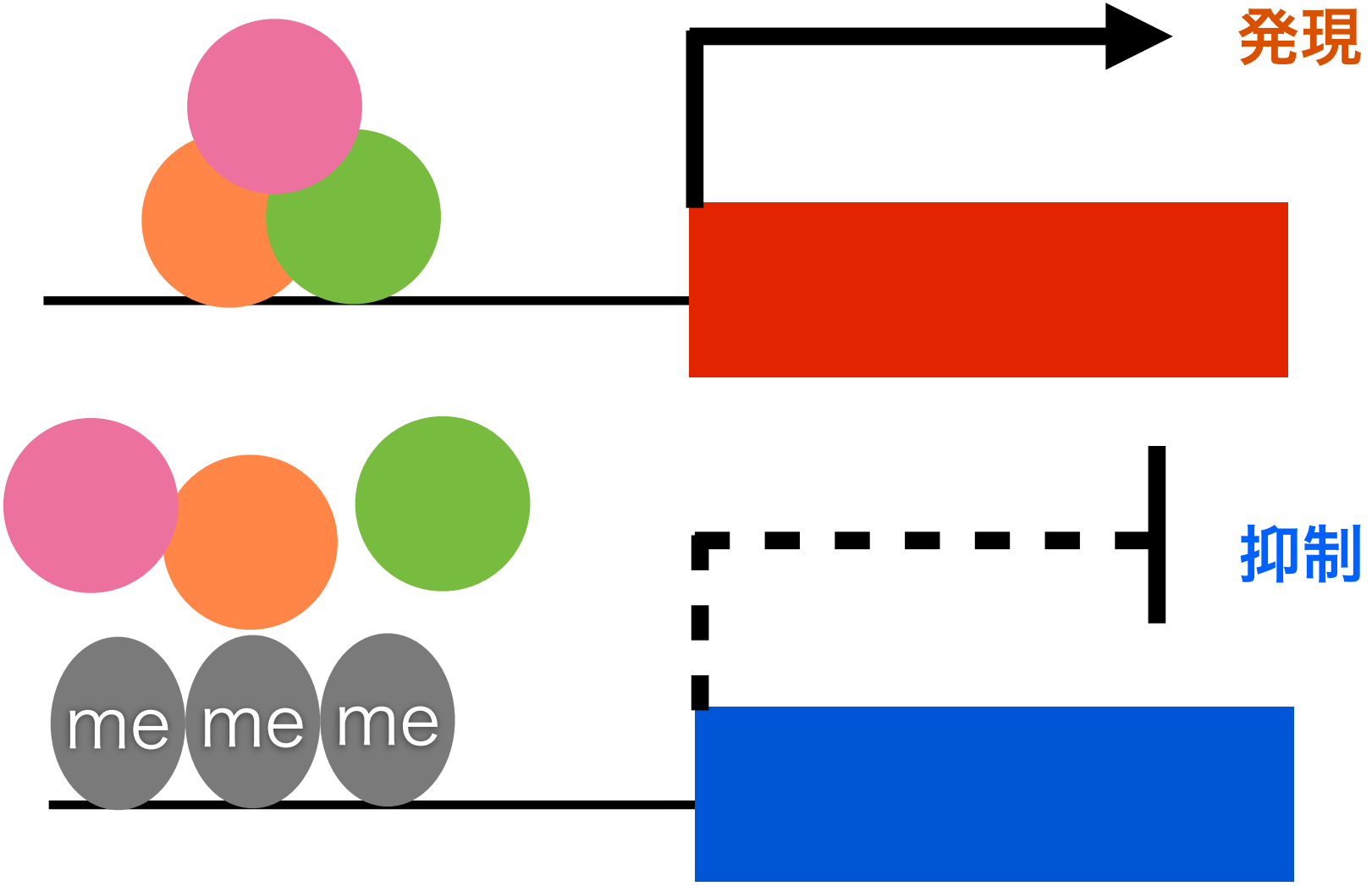
H3K4me3, active

H3K9me2/3, repressive

H3K27me3, repressive

H3K36me3, active

# DNAメチル化は遺伝子発現を抑制する



# なぜエピゲノムを調べるのか？

# どう調べるのか？

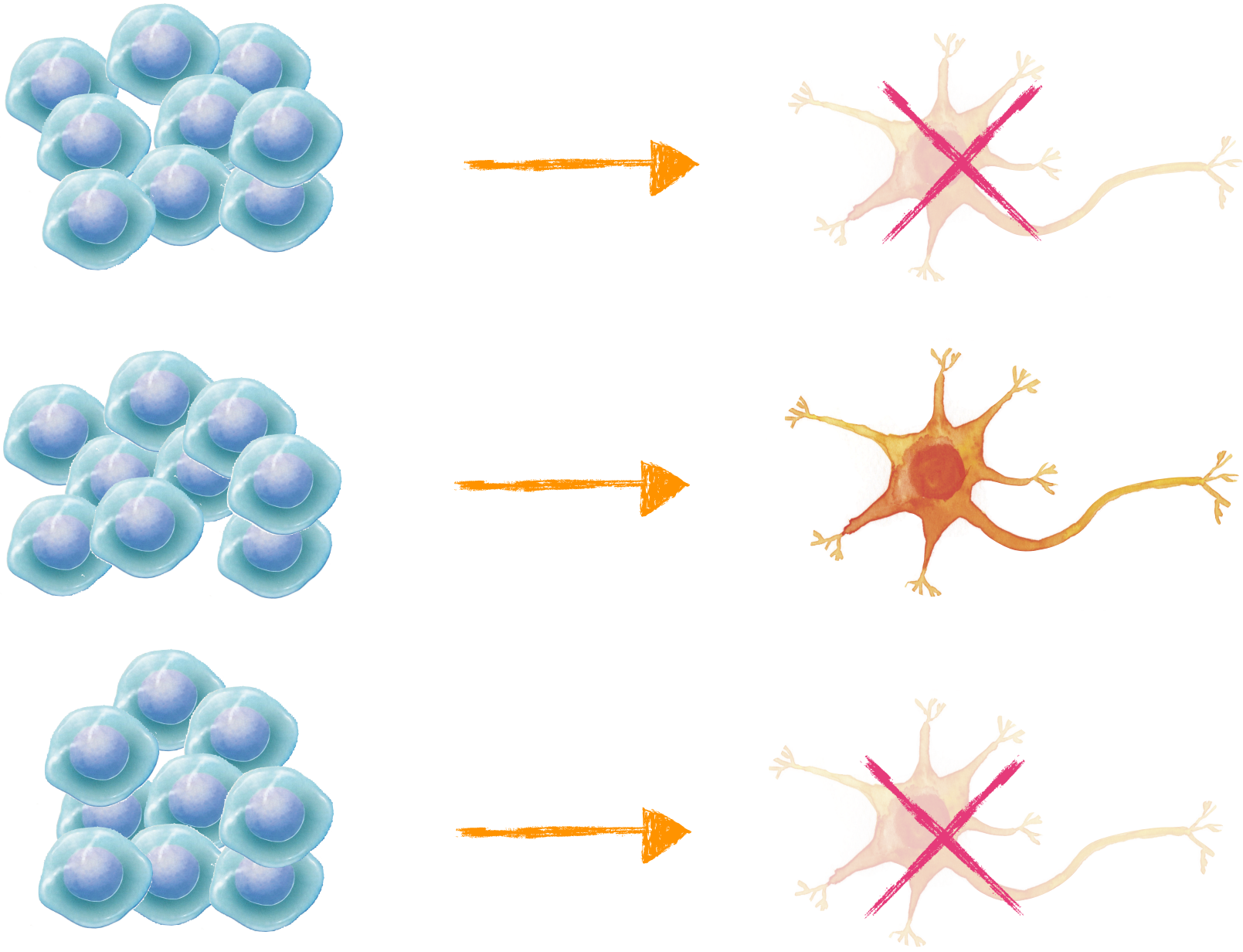
# 次世代エピゲノム解析

# なぜエピゲノムを調べるのか？

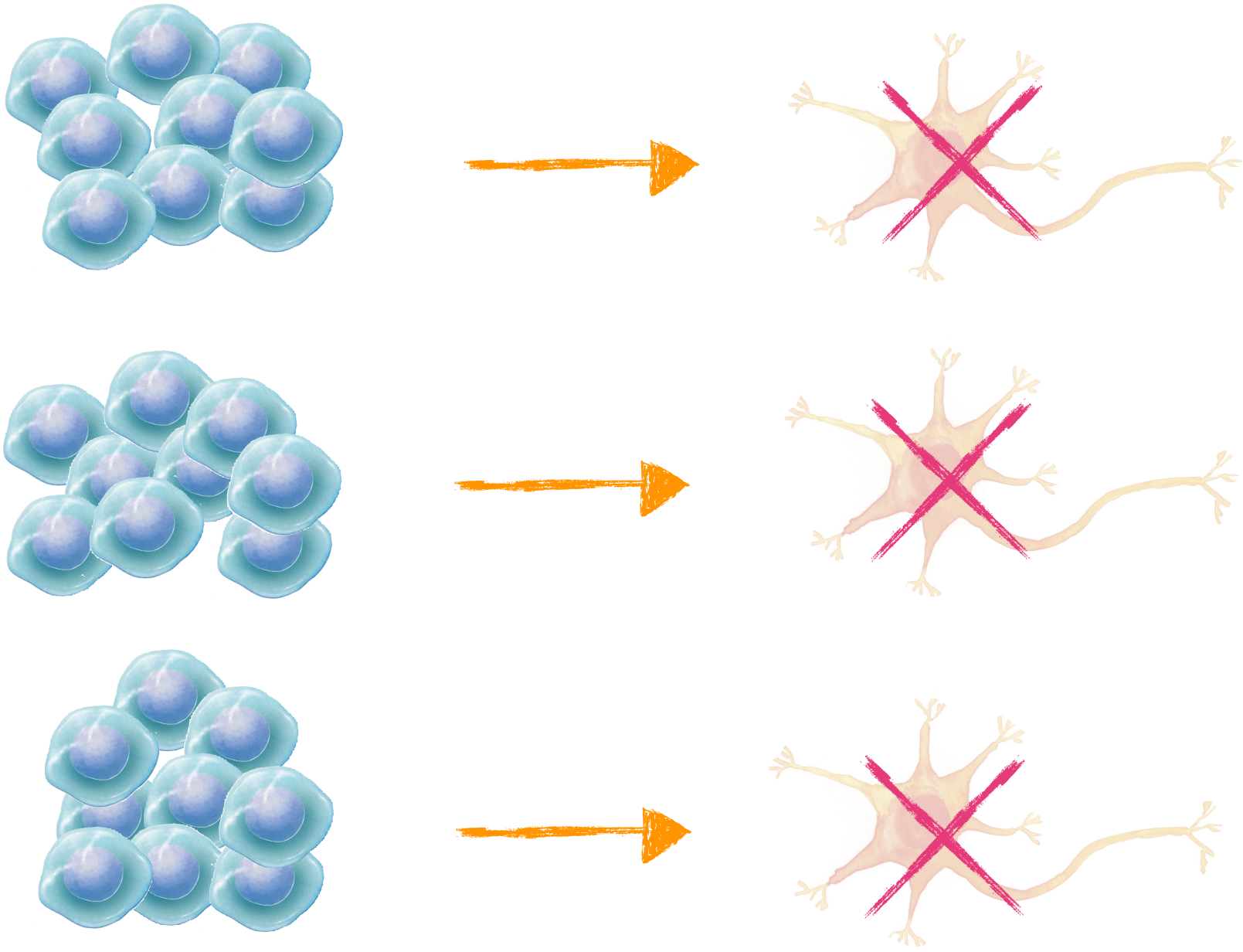
どう調べるのか？

次世代エピゲノム解析

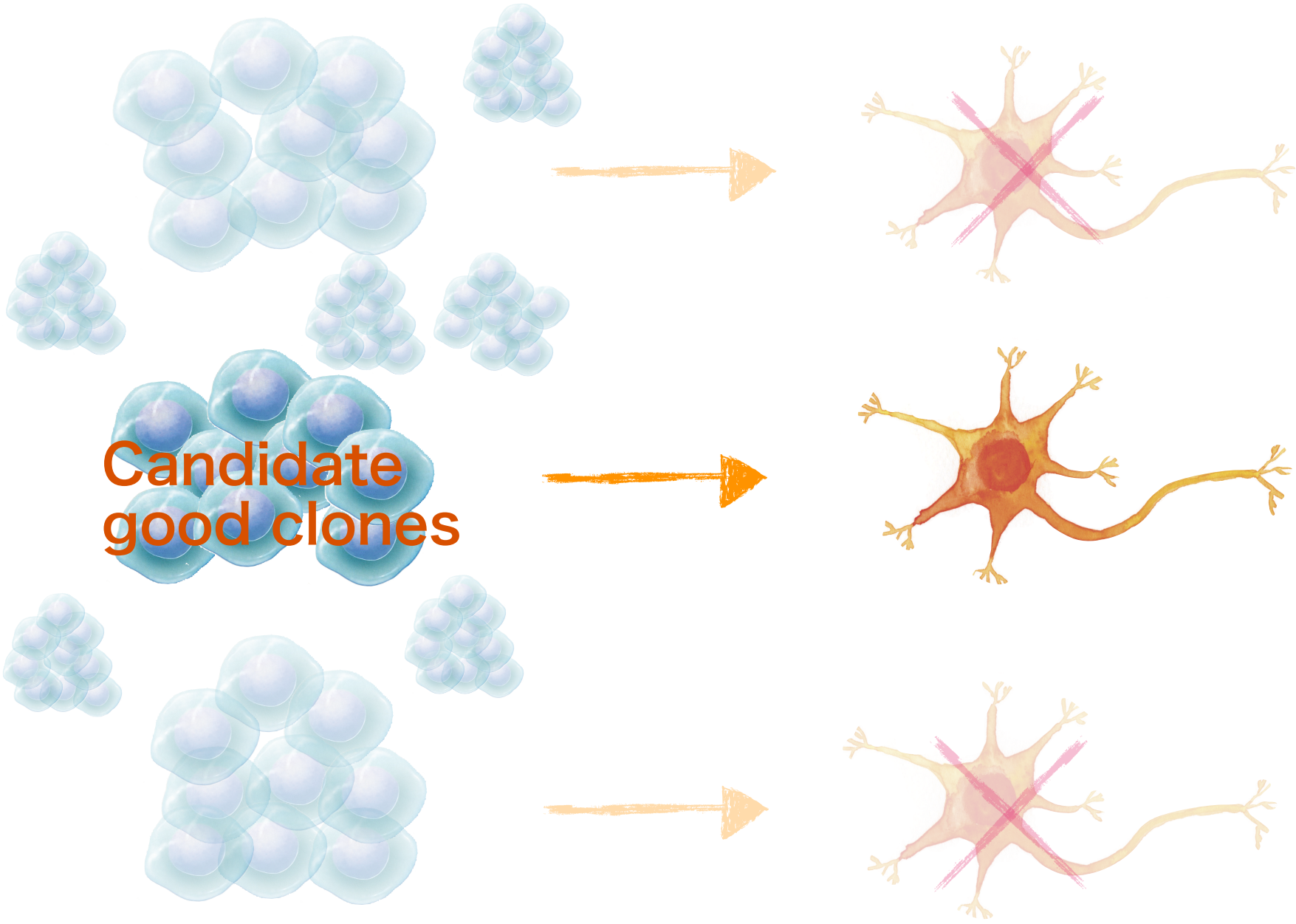
# 30 days!



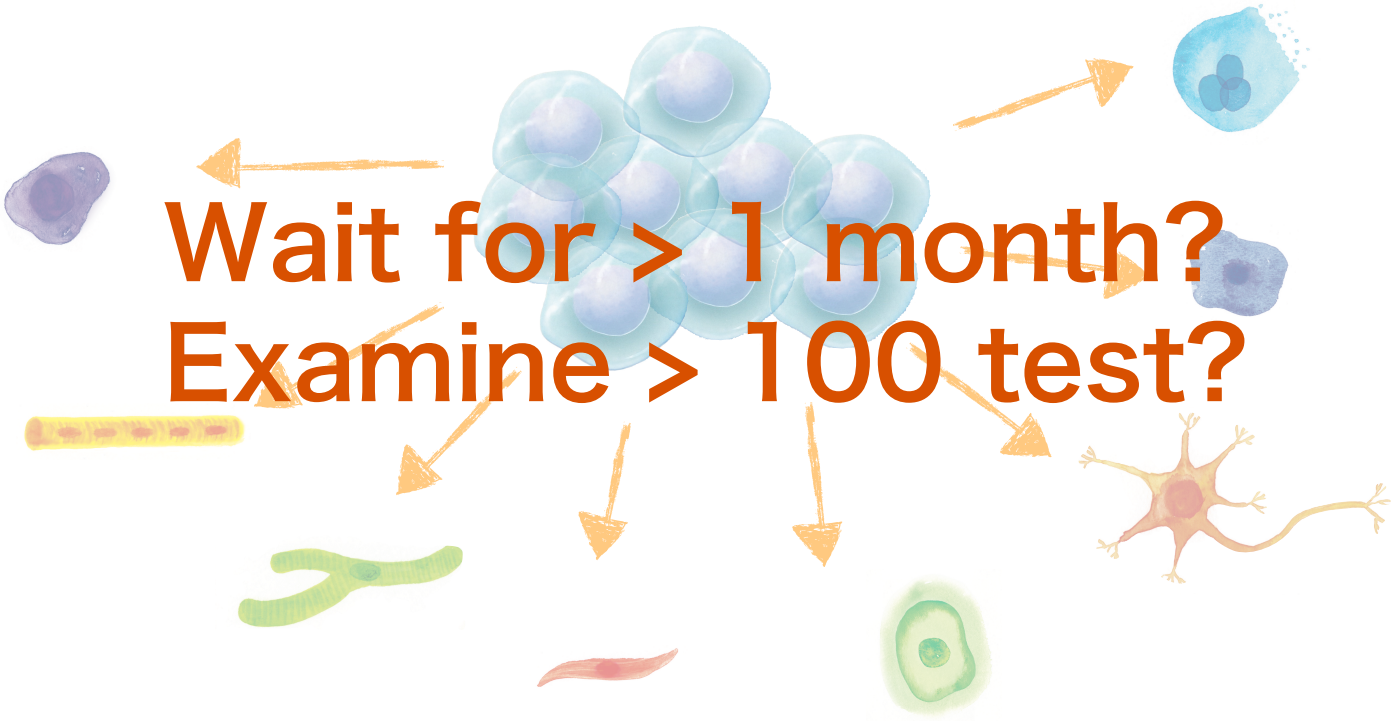
# Need to Re-try?



# Only Good Clones!



- Taking a long time to test differentiation
- Big labor to test of iPSC clones for multiple cell type
- Heterogeniety in cell populations of iPSC



Differentiation

30 days

10-50 clones

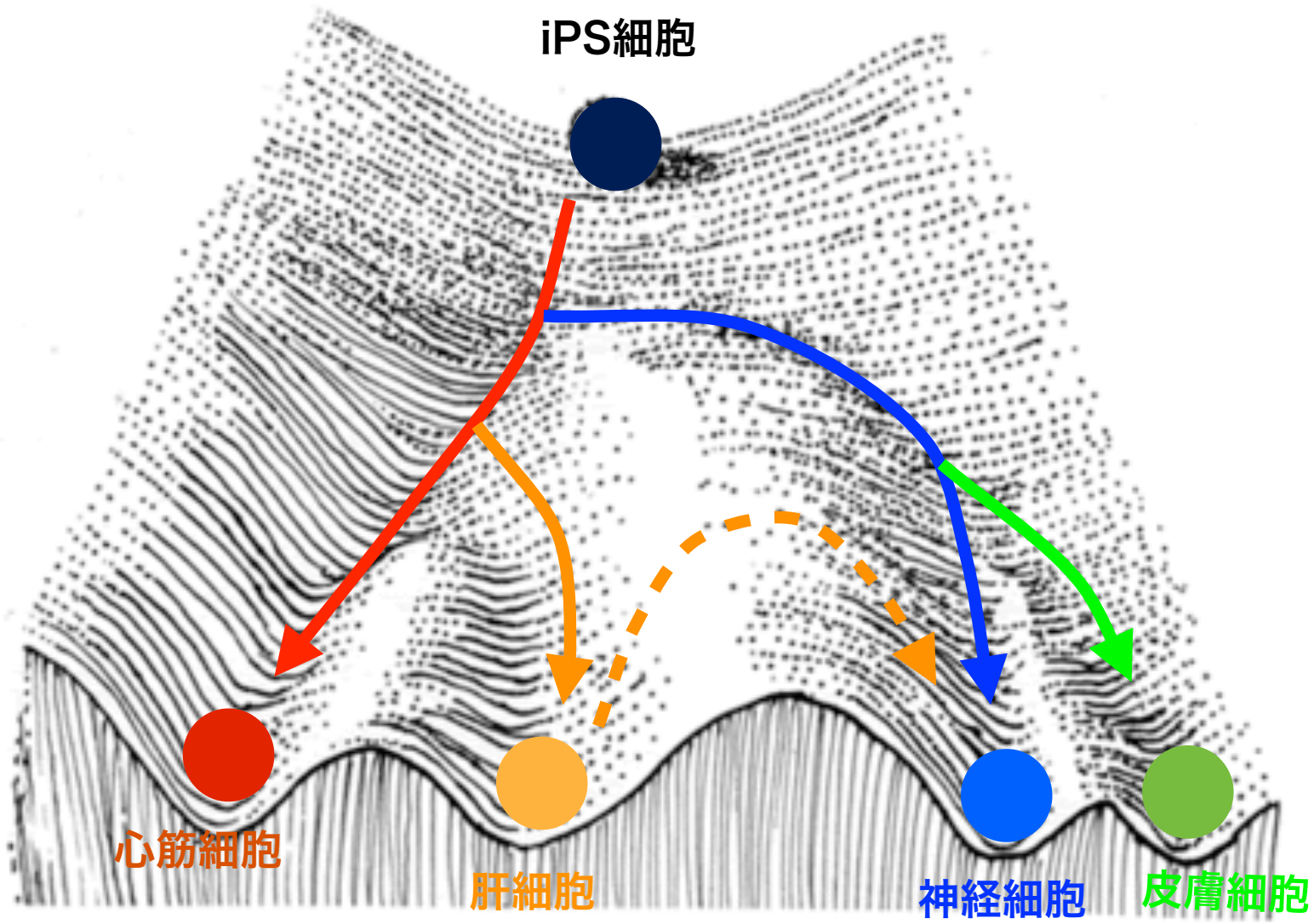
Epigenome typing

2-4 days

>100 clones

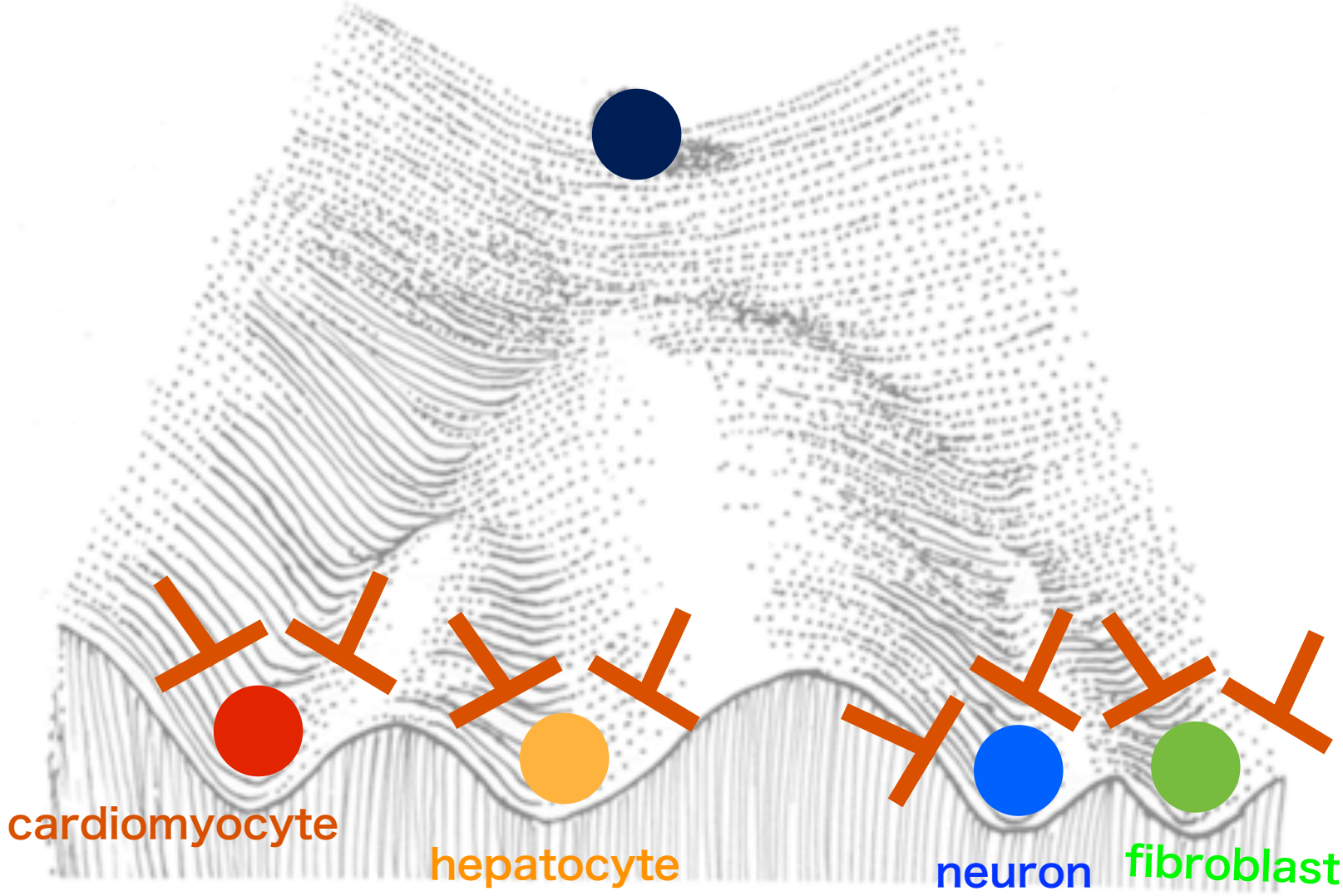
# エピジェネティック
# リプログラミング

# Epigenome Makes 220 cell types!



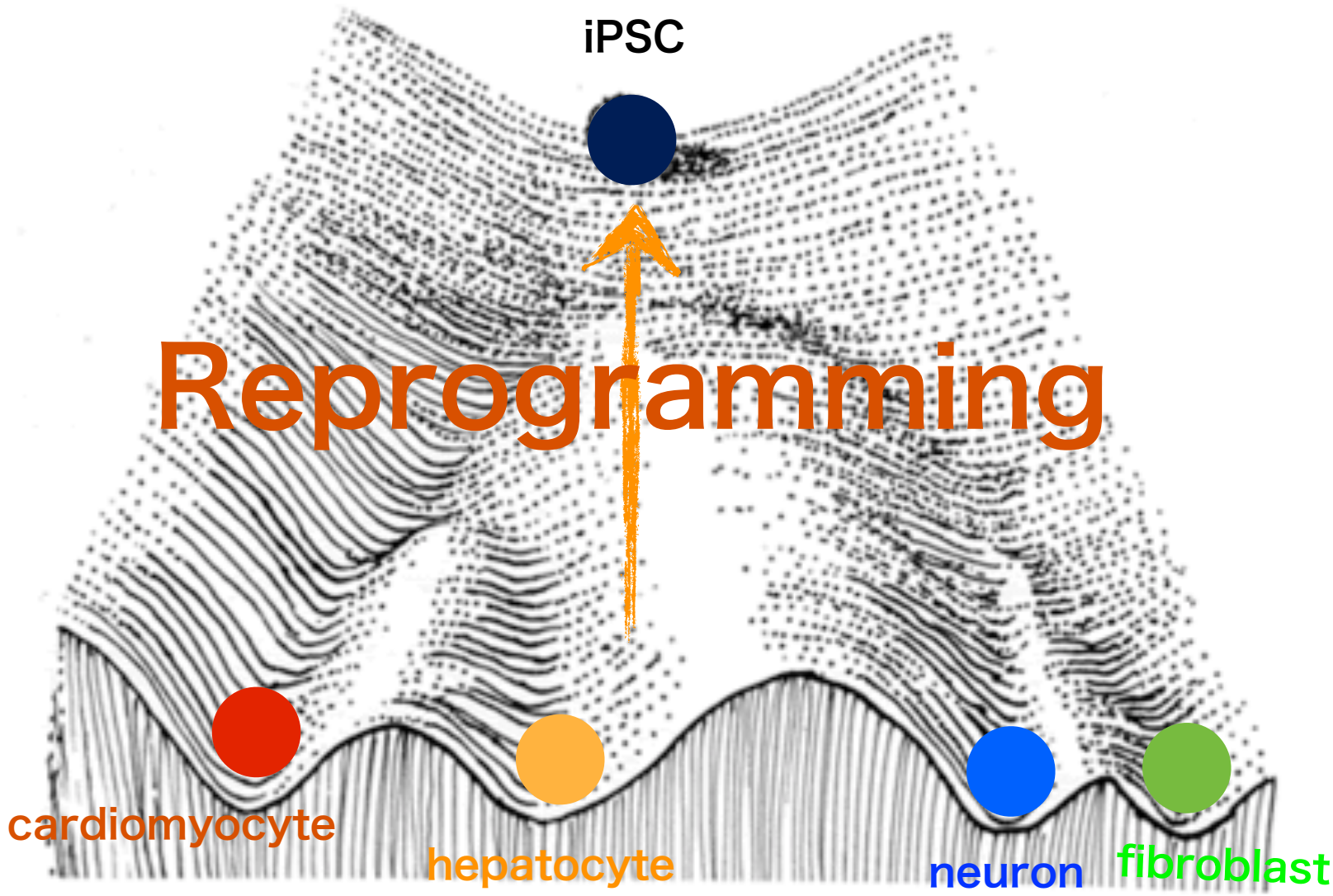

Waddington's Model

# Cell Fate Is Protected by Epigenome




Waddington's Model

# How to Break the Epigenetic Barrier?




Waddington's Model

エピジェネティクスは
分化を制御する
メカニズムである

特にDNAメチル化は分化の制御に重要なメカニズムである

プロモータ領域がDNAメチル化を受けた遺伝子の発現は

**完全に抑制**される

DNAメチル化／脱メチル化は

**DNA複製時**のみ

行われる（受精直後を除く）

DNAメチル化は安定な

マークである

Nature 2010

# Epigenetic memory in induced pluripotent stem cells

K. Kim[1], A. Doi[2], B. Wen[2], K. Ng[1], R. Zhao[1], P. Cahan[1], J. Kim[3], M. J. Aryee[4], H. Ji[2], L. I. R. Ehrlich[5]†, A. Yabuuchi[1], A. Takeuchi[1], K. C. Cunniff[1], H. Hongguang[1], S. Mckinney-Freeman[1], O. Naveiras[1], T. J. Yoon[6]†, R. A. Irizarry[2], N. Jung[2], J. Seita[5], J. Hanna[7], P. Murakami[2], R. Jaenisch[7], R. Weissleder[6], S. H. Orkin[3], I. L. Weissman[5], A. P. Feinberg[2] & G. Q. Daley[1]

**体細胞初期化は**
**エピジェネティックな状態を**
**完全に解除していない？**

# なぜエピゲノムを調べるのか？

# iPS細胞の分化指向性を予測する

なぜエピゲノムを調べるのか？

# どう調べるのか？

次世代エピゲノム解析

infinium assay

sequence-capture-based deep seq.

# DNAメチル化を用いた iPS細胞のエピゲノム判定法を確立する

# 分化指向性に関係する
# DNAメチル化を同定する

Infinium DNA methylation assay 27K (illumina社)

- 易分化性iPS細胞　４株
- 難分化性iPS細胞　３株

# Infinium Assay

by illumina

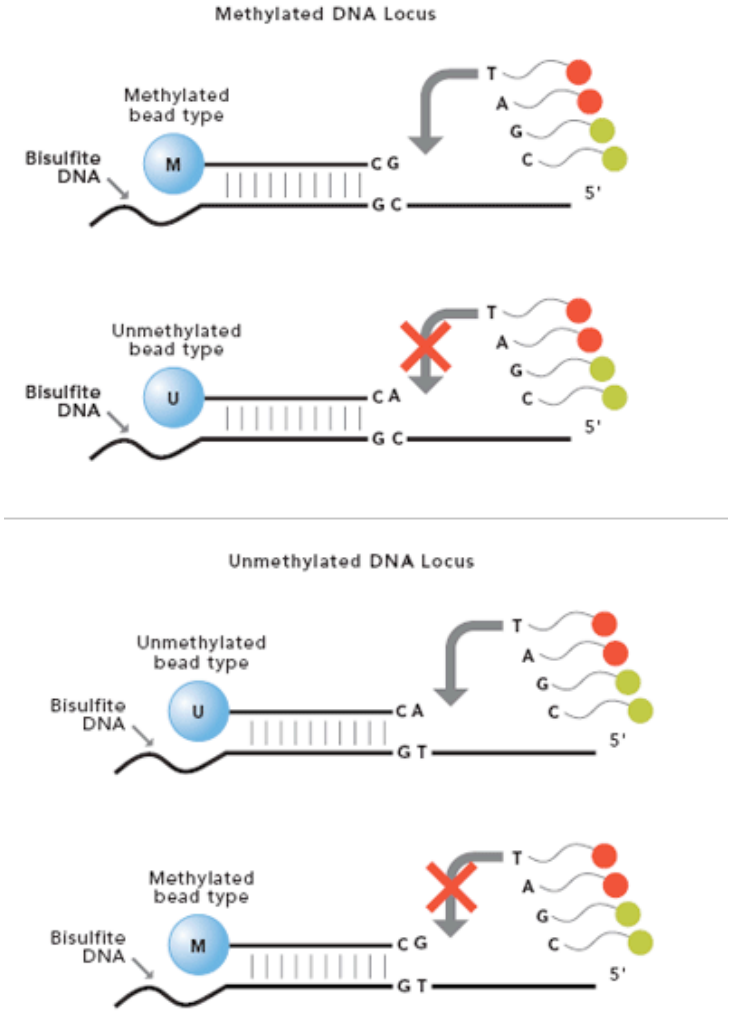

genotype-based assay
using microarray

450K probes

# HumanMethylation 450K Beads Chip

C → T

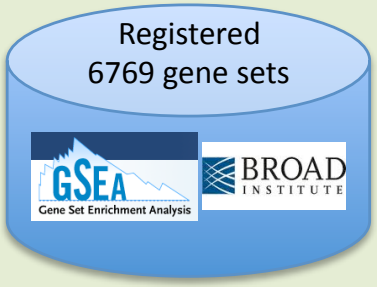
mC → mC

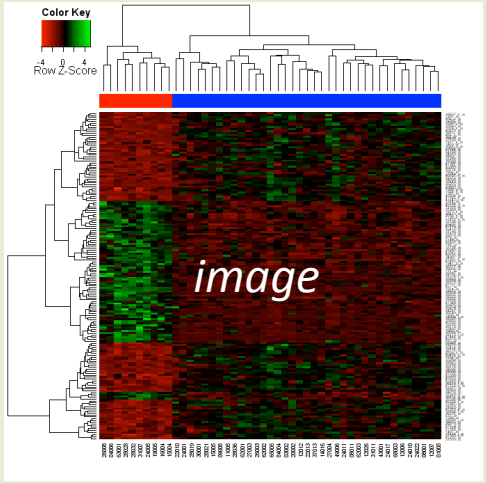


# アプローチ

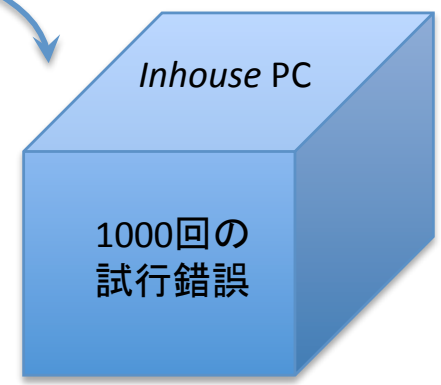
## Gene Set Enrichment Analysis

手持ちのマイクロアレイデータなどを既知の遺伝子セットと比較し、実験データの表現系を推測する方法

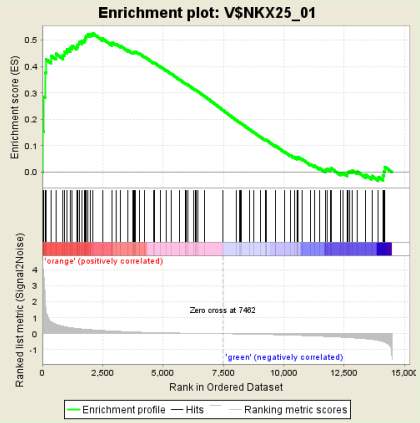


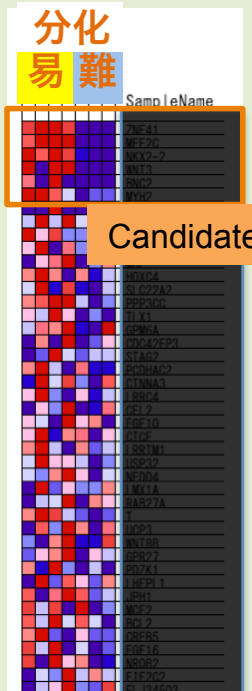




Gene expression by microarray
DNA methylation measured by Infinium




GO解析と異なり、データはすべて投入できる。
また、参照とするデータが他のマイクロアレイの結果のものも含まれる。




遺伝子W

Candidate sleeping mark

メチル化

非メチル化

## 結果

標的分化細胞で遺伝子WがDNAメチル化されたiPS細胞は易分化性である。

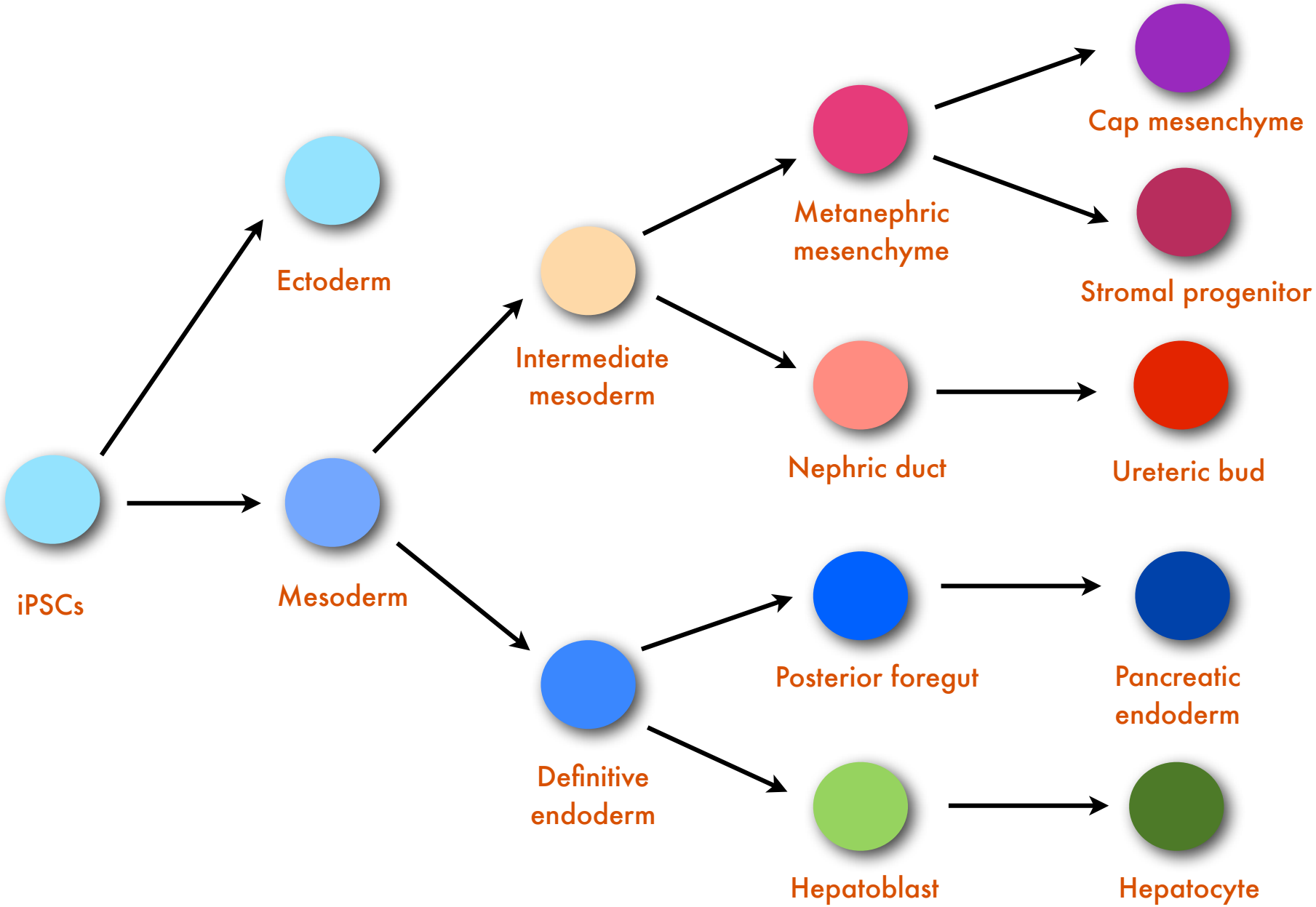
# 遺伝子WのDNAメチル化状態はiPS細胞の品質を予測しうる

## 結果

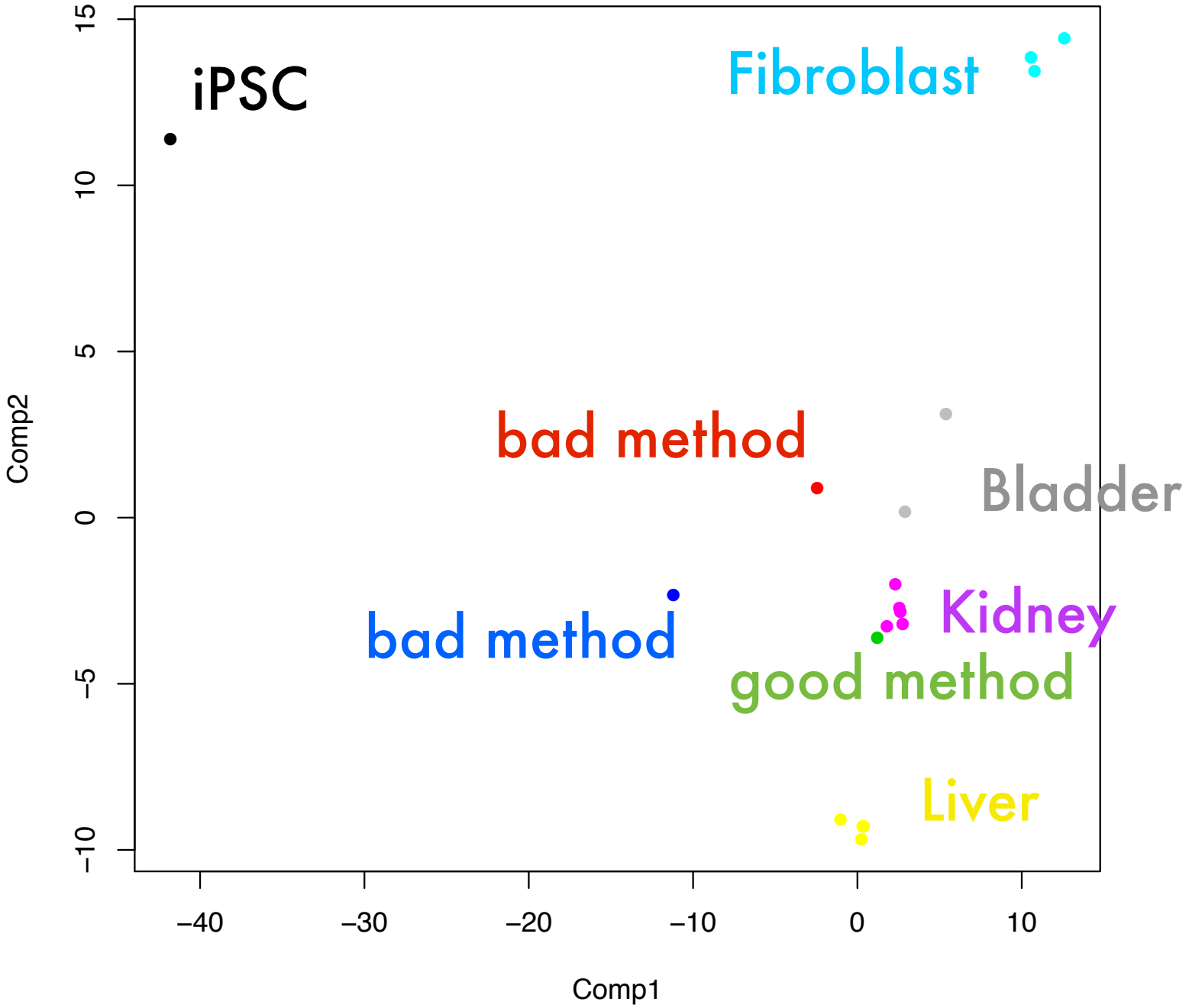
iPS細胞 → 遺伝子W阻害剤 → 標的分化細胞

# 遺伝子WのDNAメチル化は機能的にも重要である

# Differentiation into Renal Lineage



# PCA Analysis



なぜエピゲノムを調べるのか？

# どう調べるのか？

次世代エピゲノム解析

infinium assay

sequence-capture-based deep seq.

# 新規のエピゲノムマークを探索しよう！

# アプローチ

## Methyl-Seq for Deep Seq. of DMR


Fragmentation of gDNA


Adaptor ligation

Hybridize the target region with Methyl-Seq probes


Bisulfite conversion

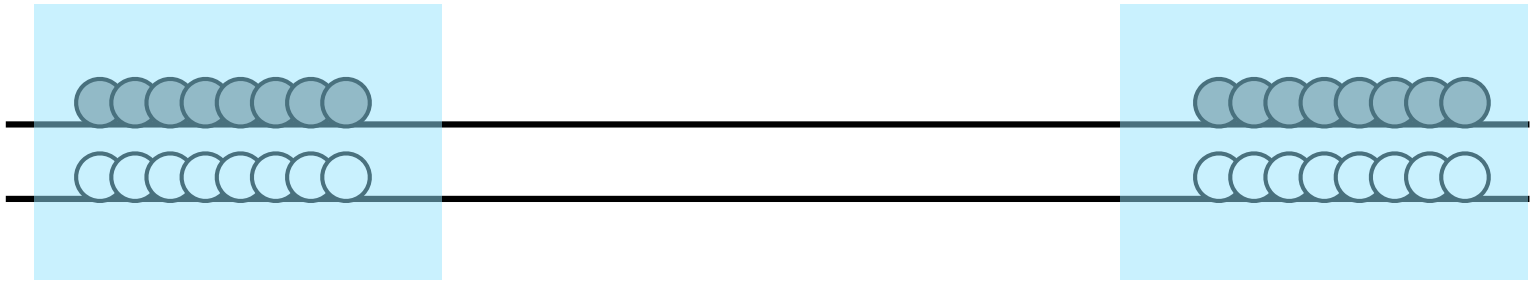
C to U/T
mC to mC

Capture the hybridized DNA using Strep. beads

Deep sequencing by HiSeq2000 (100bpX2)

# Methyl-Seq

1. Capture the target.



2. Bisulfite conversion

C → T

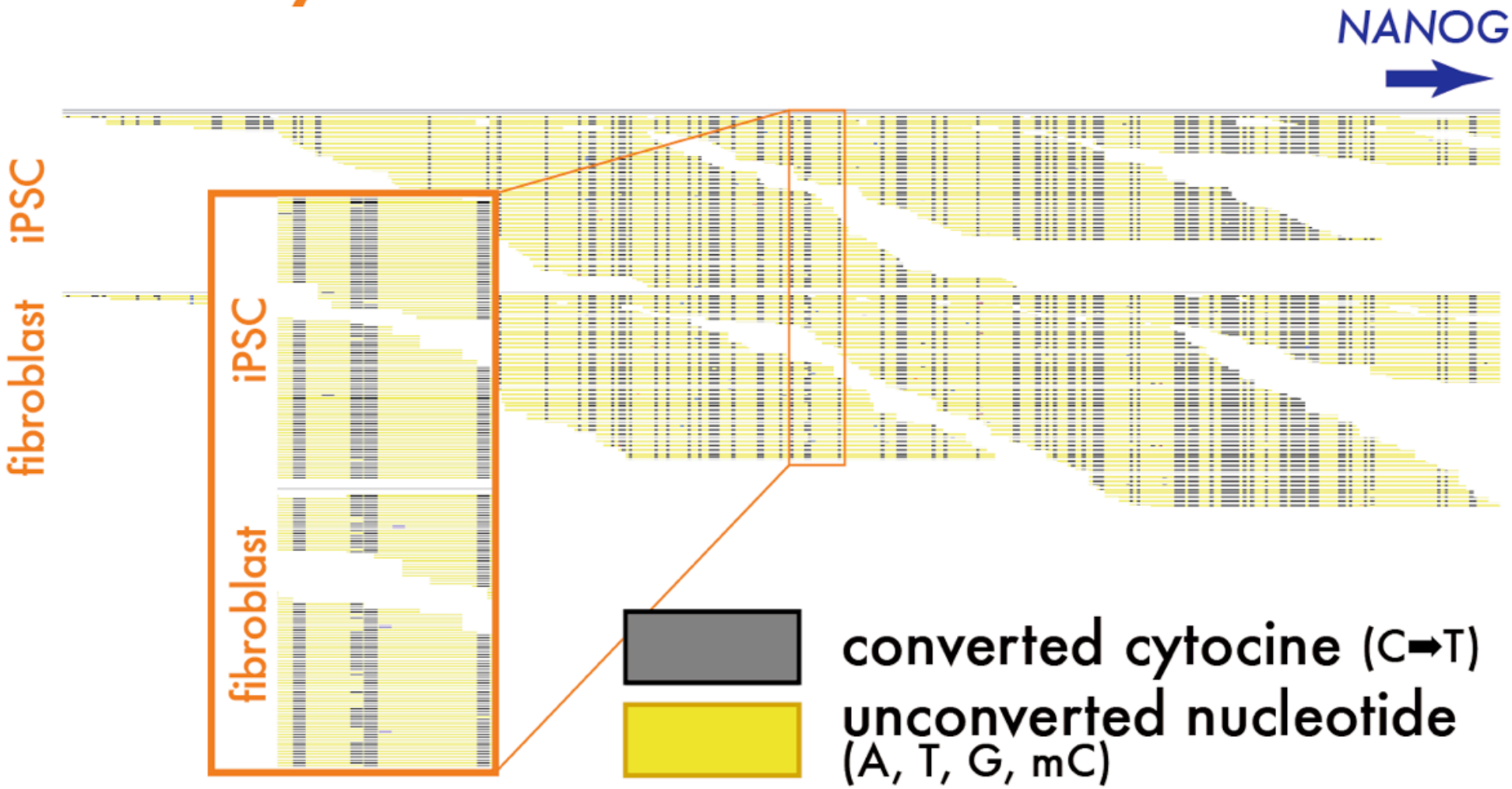
mC → mC

3. Deep sequencing

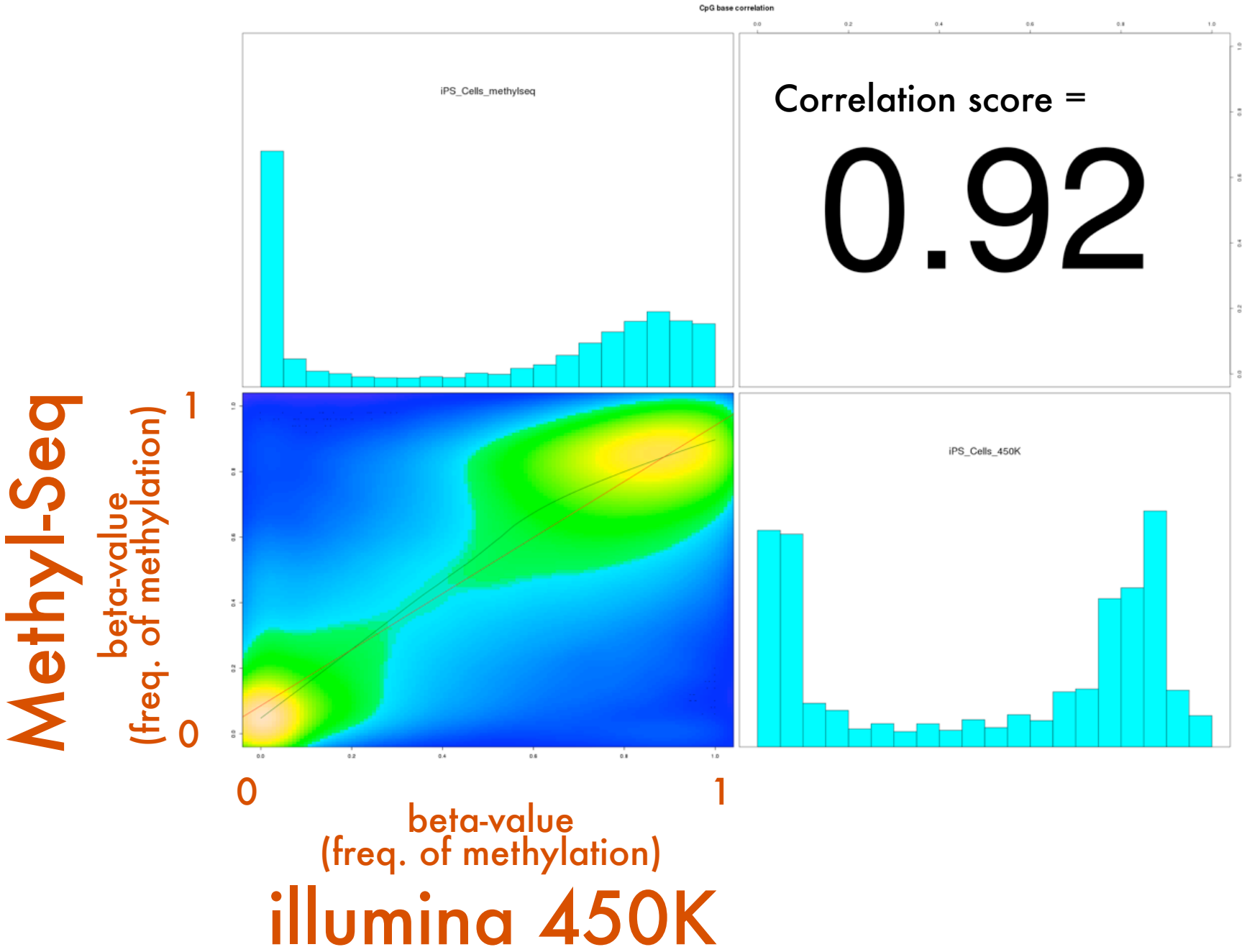
# Feature of Methyl-Seq

| Site Classification | Covered regions (bp) | CpGs covered by baits |
|---|---|---|
| CpG Islands | 19,605,556 | 1,679,870 |
| Cancer- and Tissue- Specific DMRs | 9,773,047 | 293,619 |
| GENCODE promoters | 36,974,007 | 1,272,026 |
| CpG Island shores/shelves<br>Enhancer<br>Conserved Undermethylated regions<br>(UMR; Straussman et al. 2009)<br>Ensemble regulatory features<br>Dnase I hypersensitive sites | 48,021,626 | 2,057,280 |

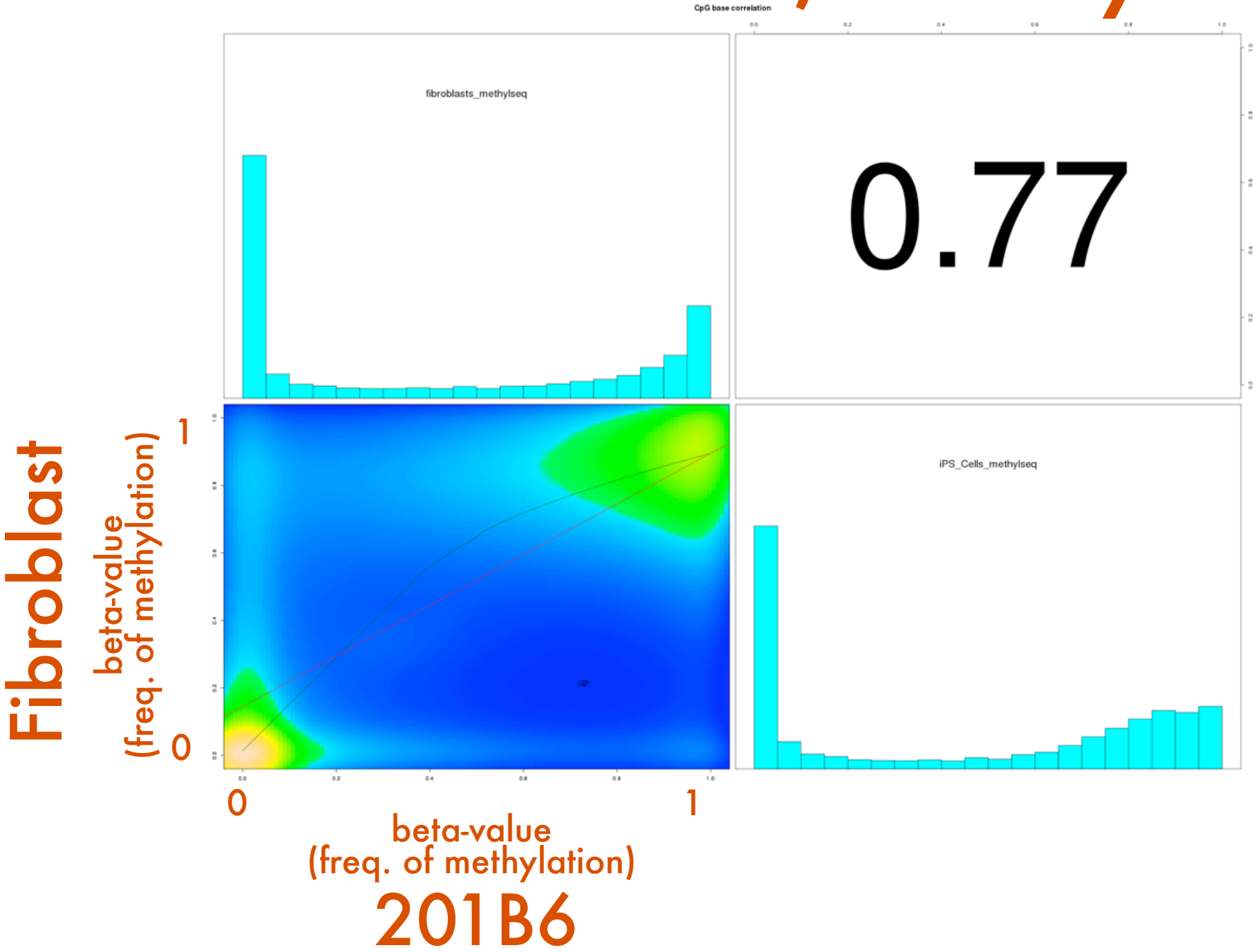
# Reproducible Detection of DNA Methylation at *NANOG* Promoter

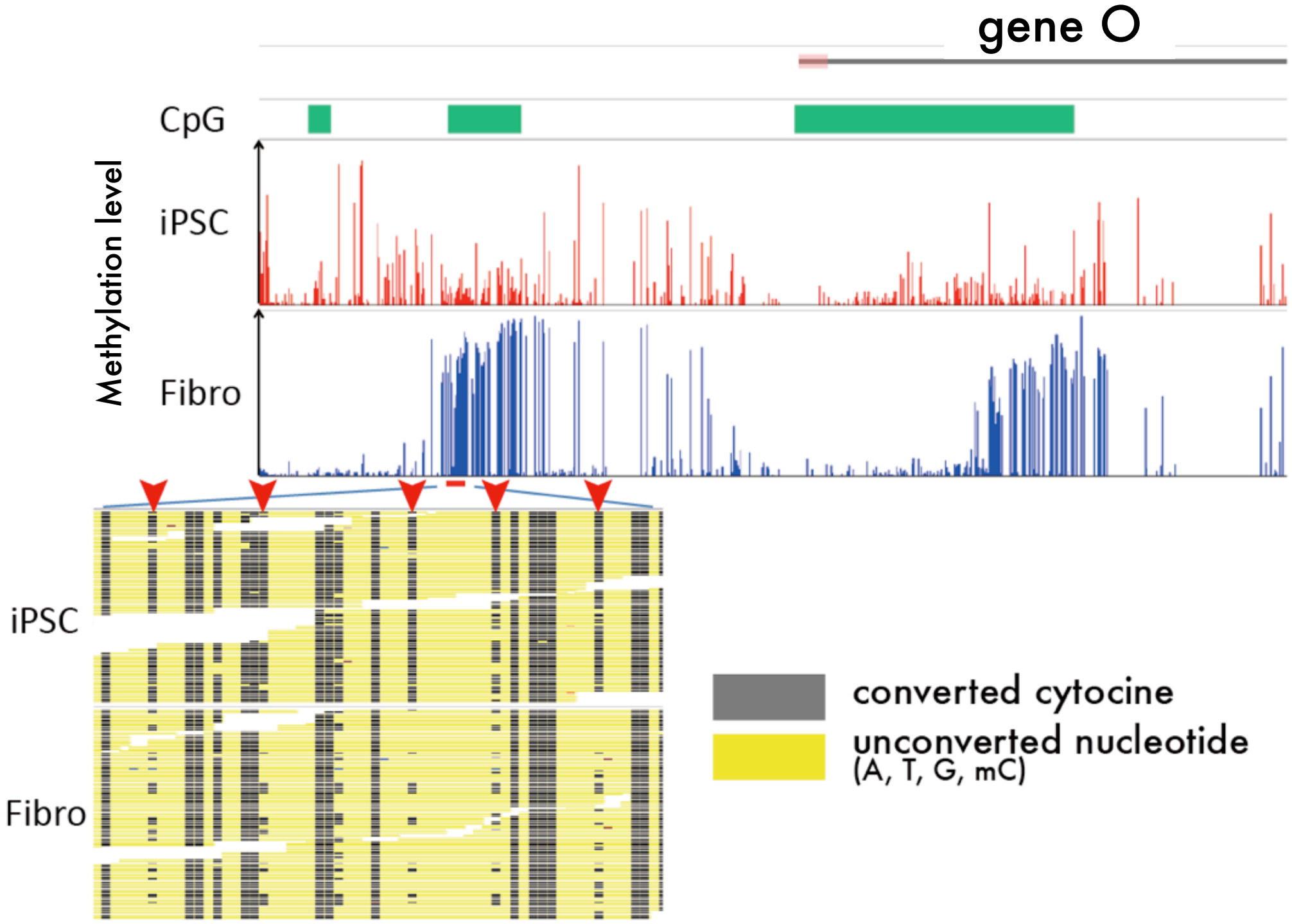


# iPSC, Methyl-Seq vs 450K

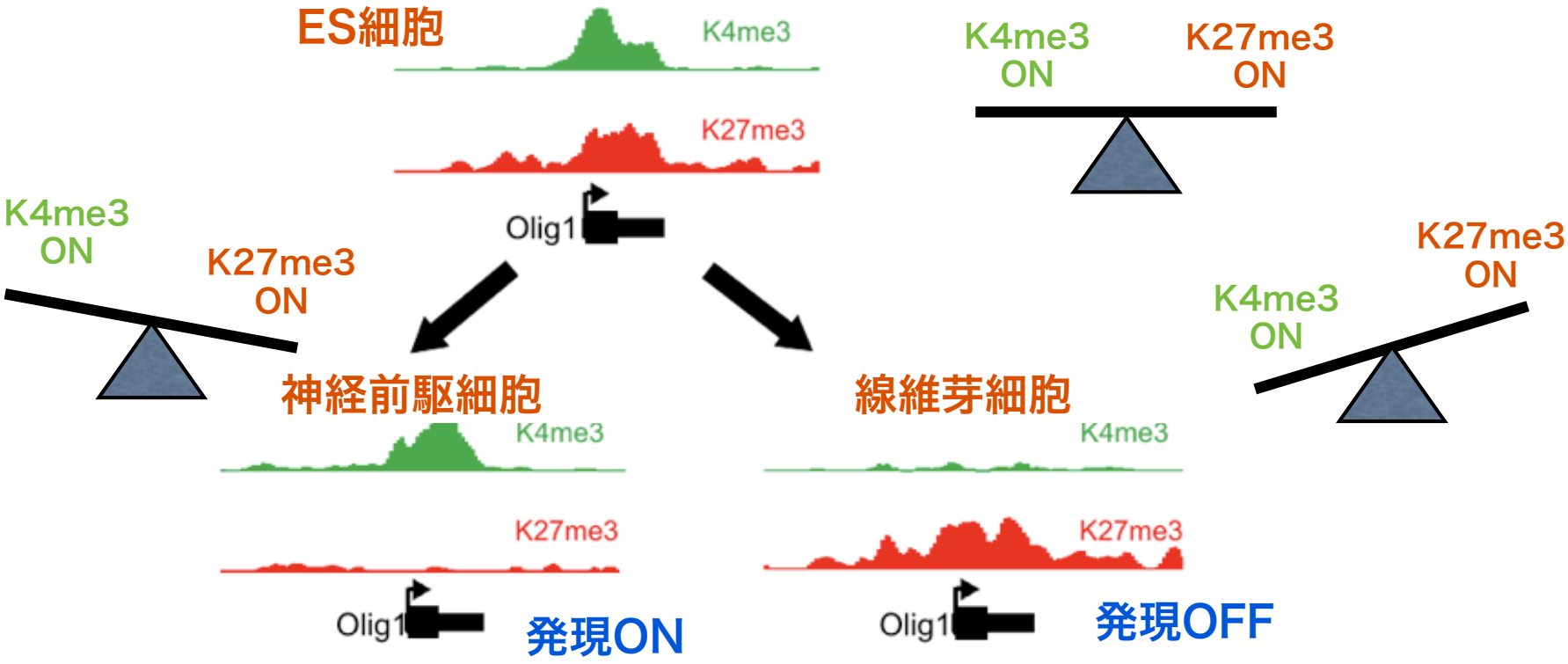


# Fibroblast vs iPSC, Methyl-Seq



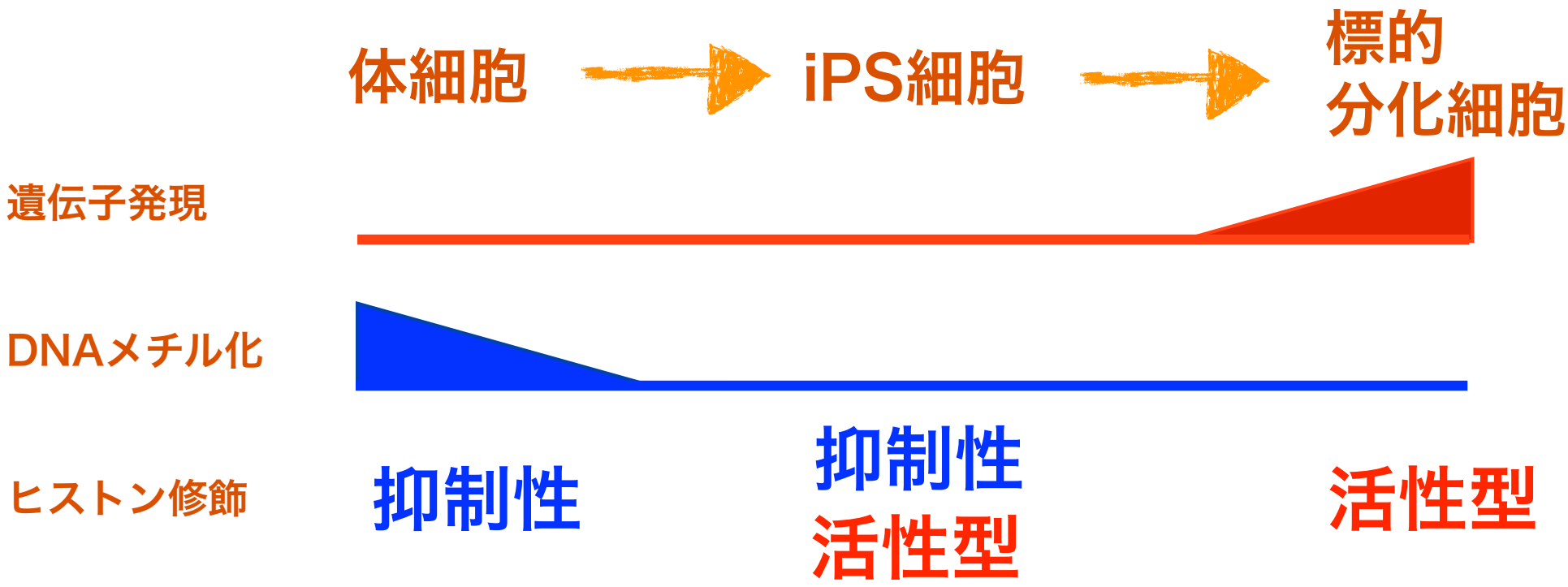

gene O
CpG
Methylation level
iPSC
Fibro
iPSC
Fibro
converted cytocine
unconverted nucleotide
(A, T, G, mC)

# 分化を制御するバイバレントな修飾




Bernstein et al, ***Cell*** 2006
Mikkelsen et al, ***Nature*** 2007
Ku, Koche, Rheinbay et al, ***PLOS Genetics*** 2009

# 結論




分化制御因子Oはバイバレントな修飾によって制御される因子である

# Diagnosis

## Finding

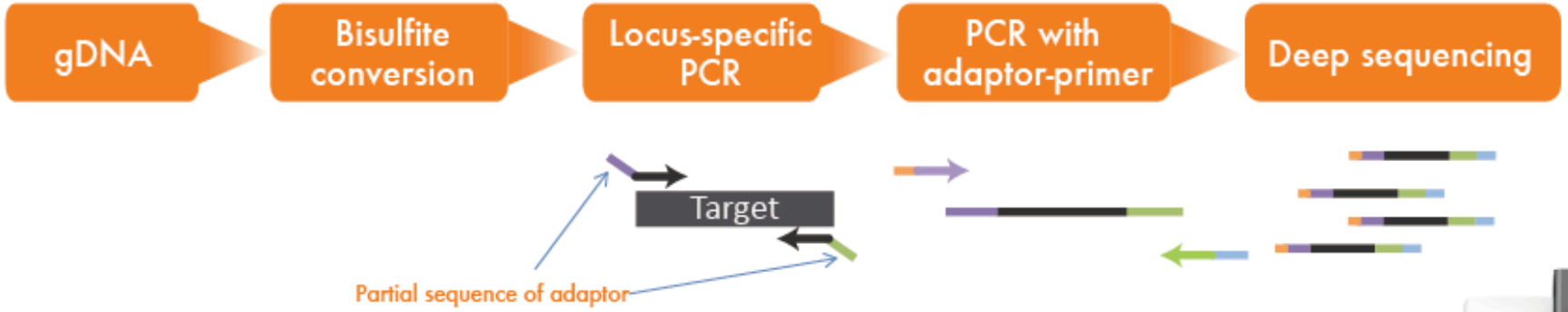
50 sample/2weeks
depth >50

## Validation

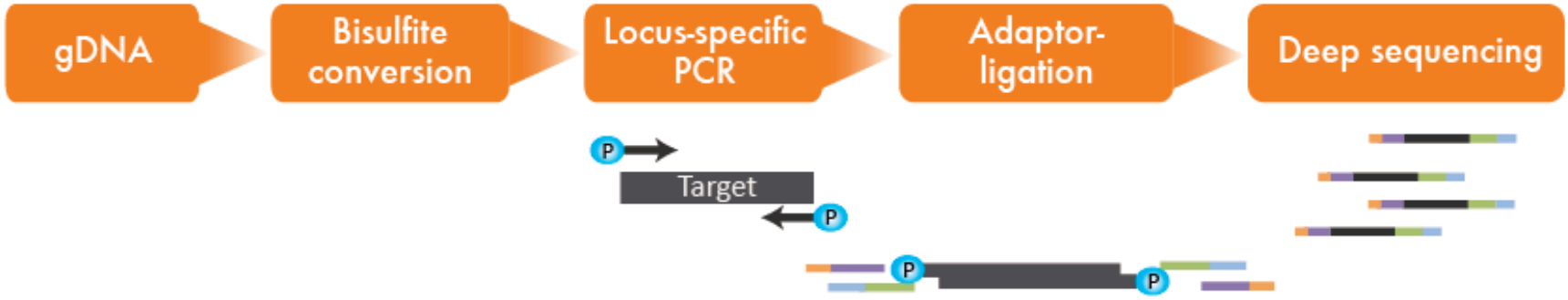
100 sample/2days
depth >10,000

# Strategy for BS-Deep Seq

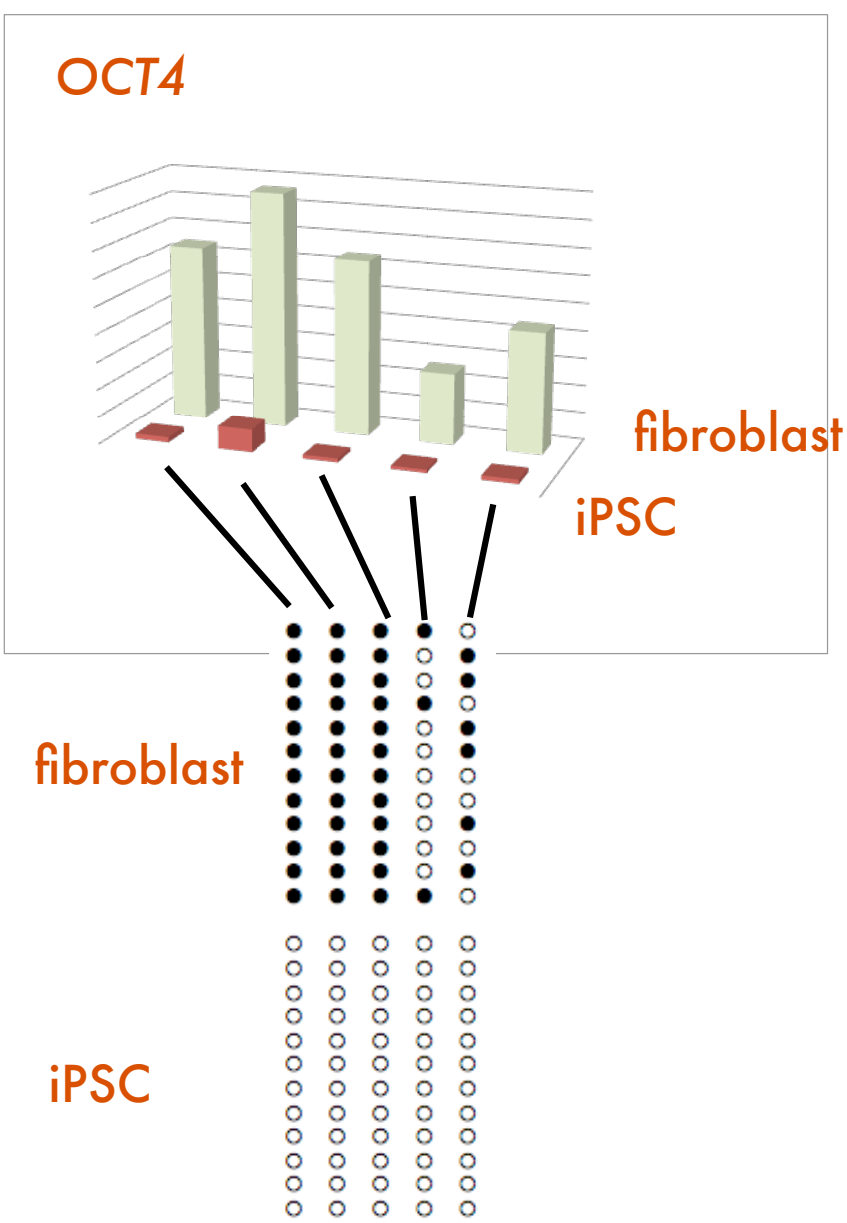
Method A, Bisulfite-converted DNA was amplified with locus specific primers, followed by PCR with adaptor-sequence

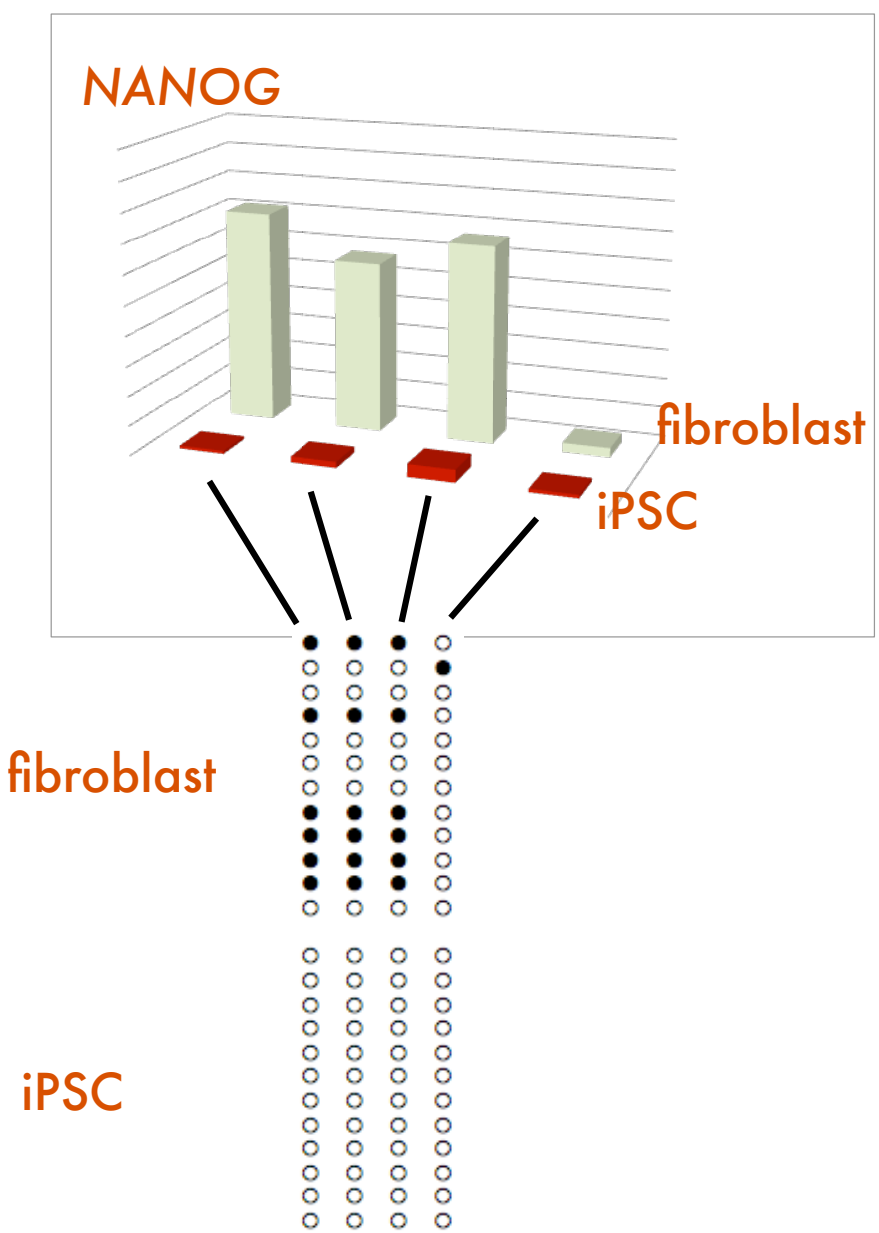



Method B, Bisulfite-converted DNA was amplified with locus specific primers, followed by ligation with adaptor-sequence



# Deep Bisulfite Seq



# High-throughput analysis of DNA methylation available

Screening by HiSeq

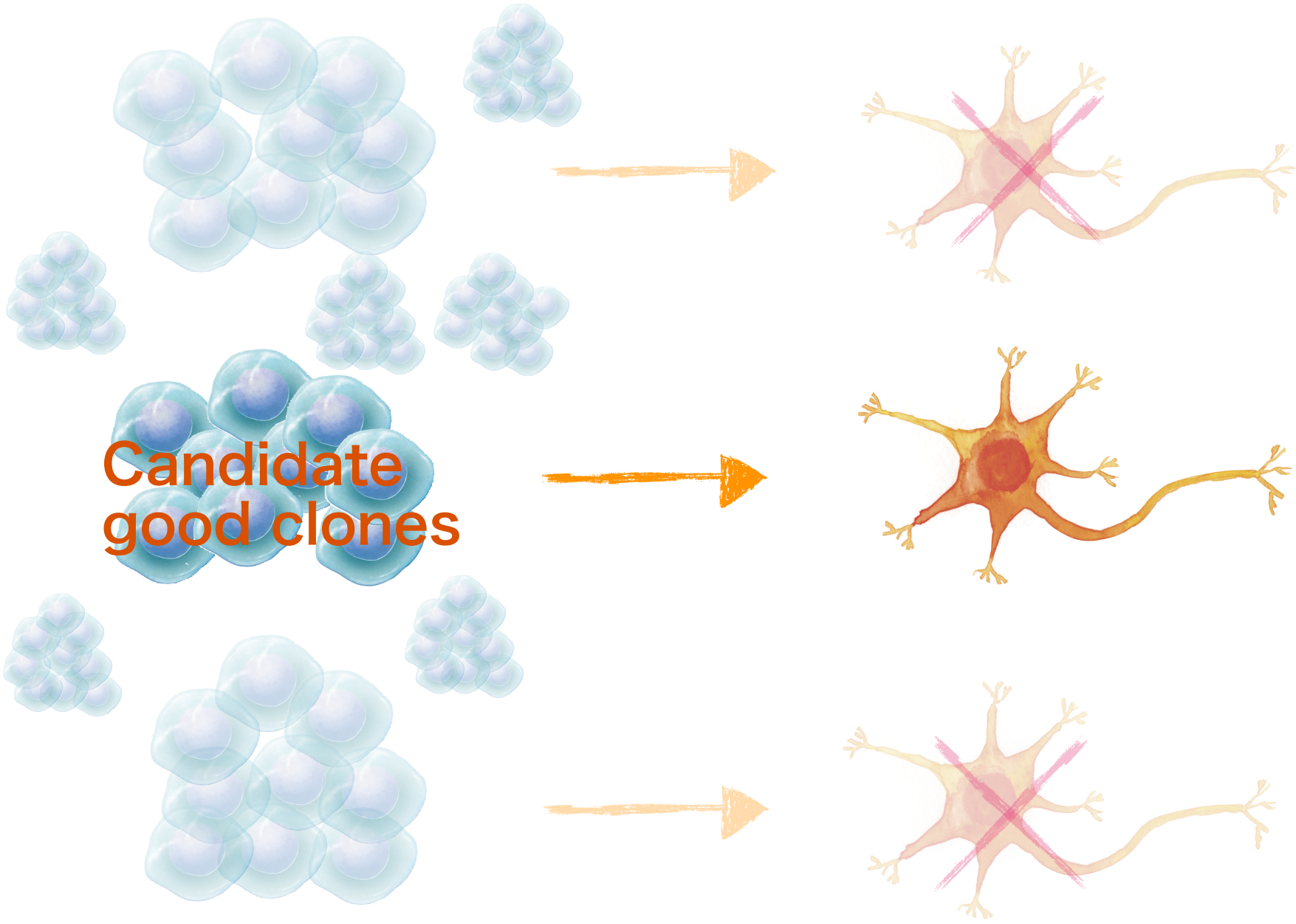
Validation by MiSeq

# DNA Methylation Predicts The Future

DNA Methylation can be used as

# a sleeping mark

# Only Good Clones!



なぜエピゲノムを調べるのか？

どう調べるのか？

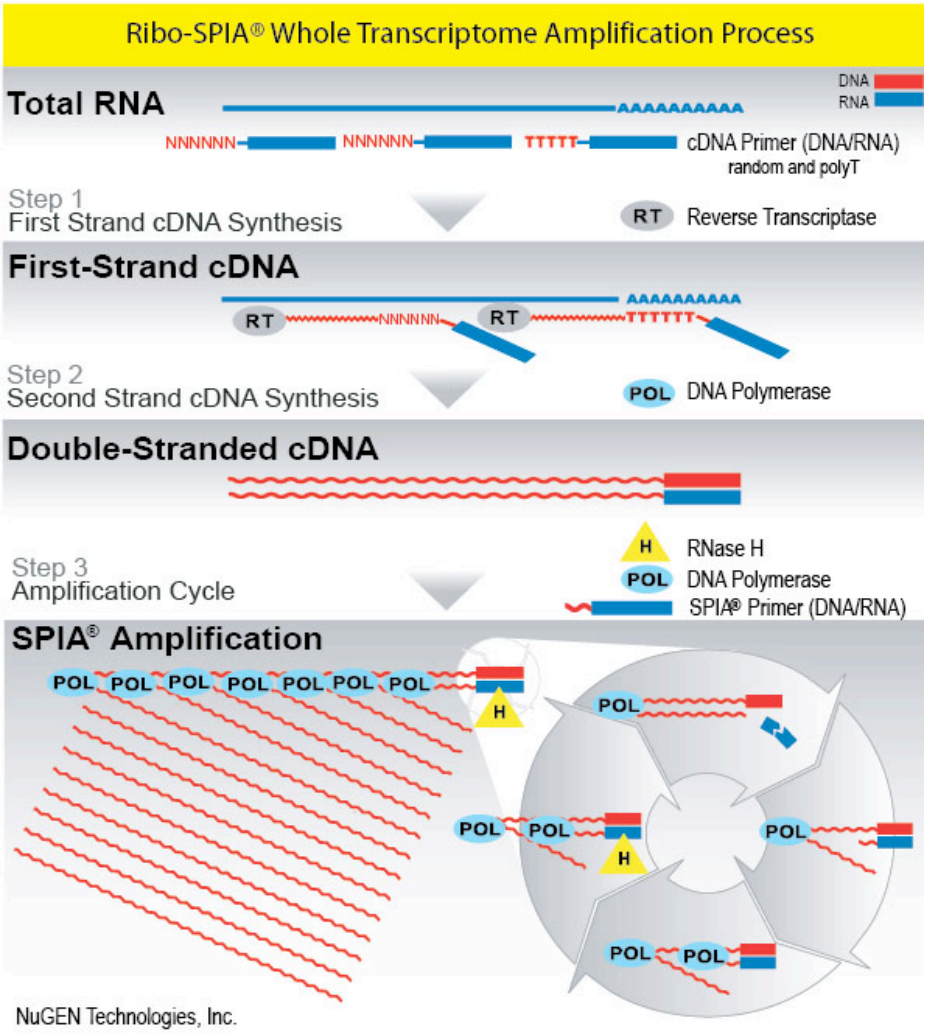
# 次世代エピゲノム解析

pico-level transcriptome

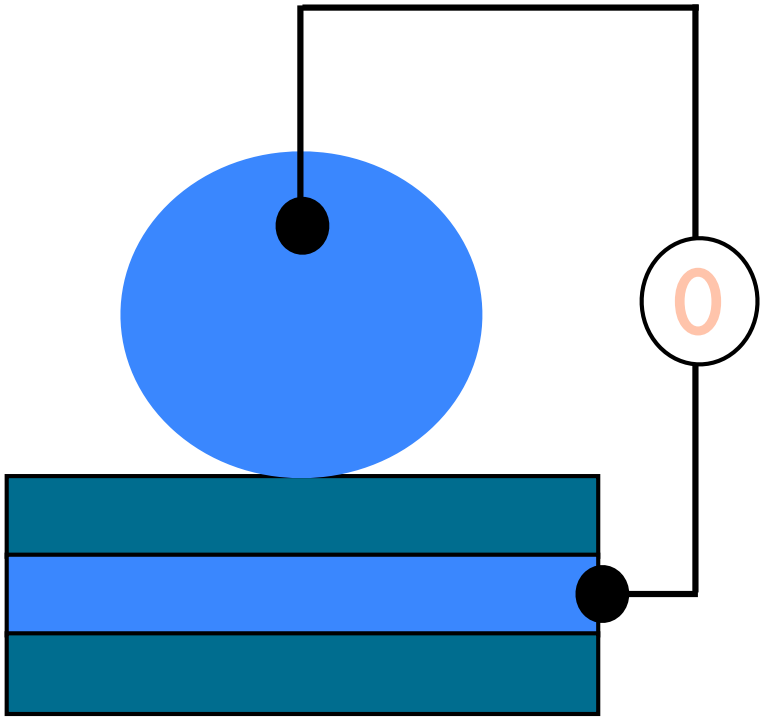
single cell transcriptome

# Linear Amplification of DNA/RNA by SPIA.

Mondrian (NuGEN)

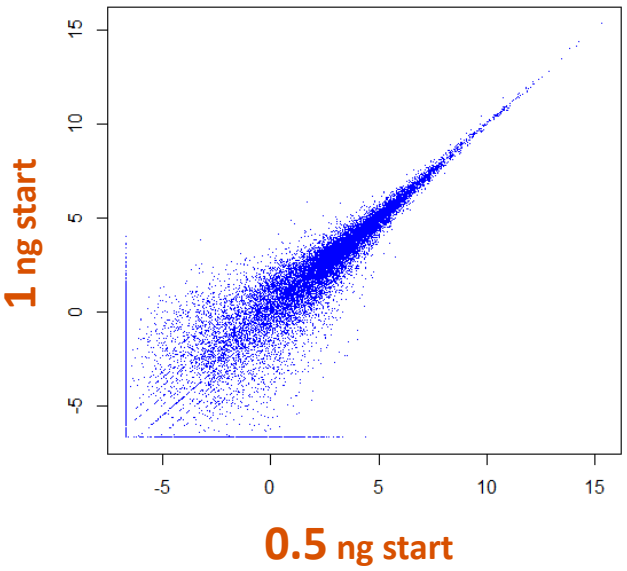


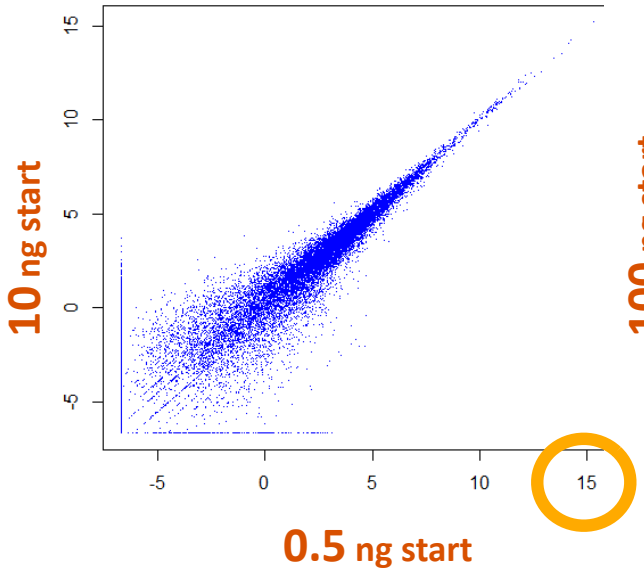
# Moving Reactant by Electrode

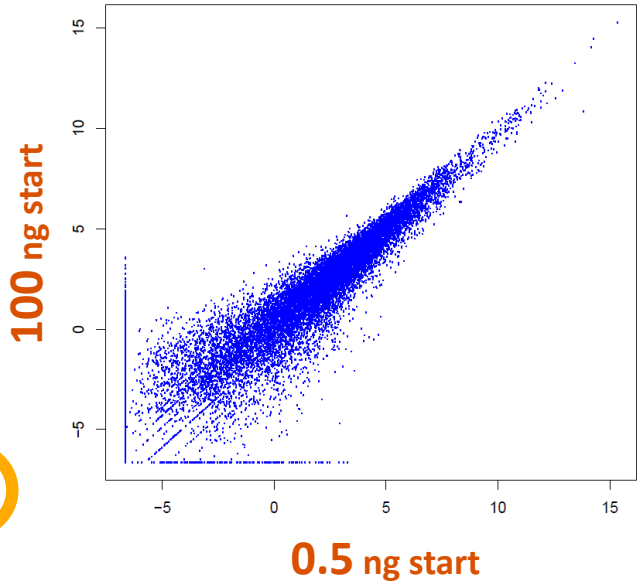



# Good Correlations in Mondrian

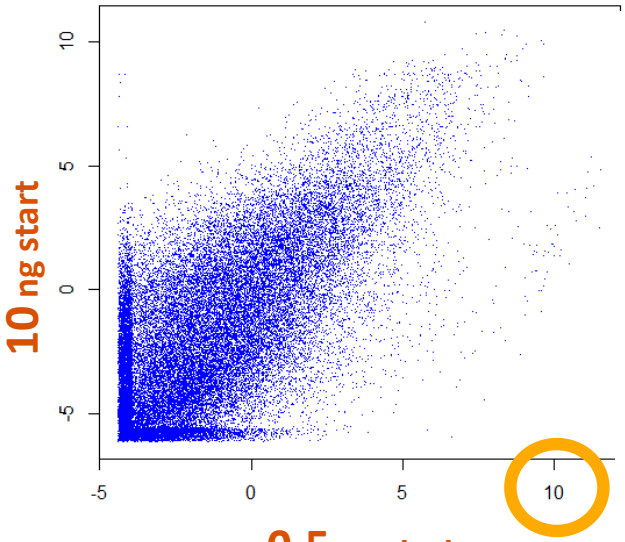
RNA-seq by Mondrian

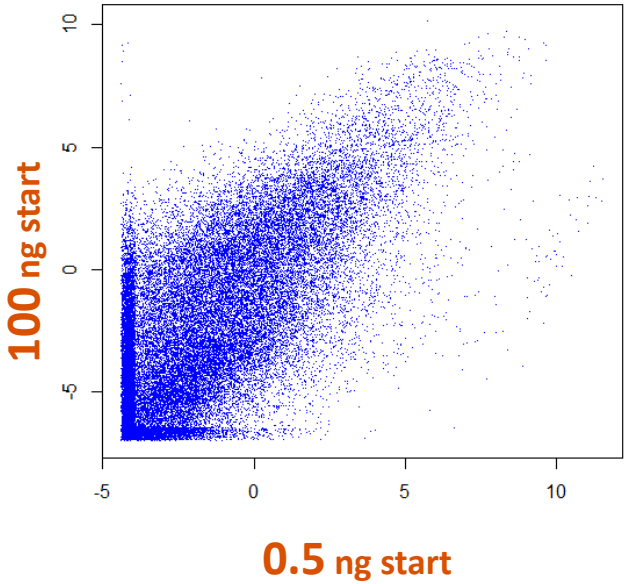



Microarray



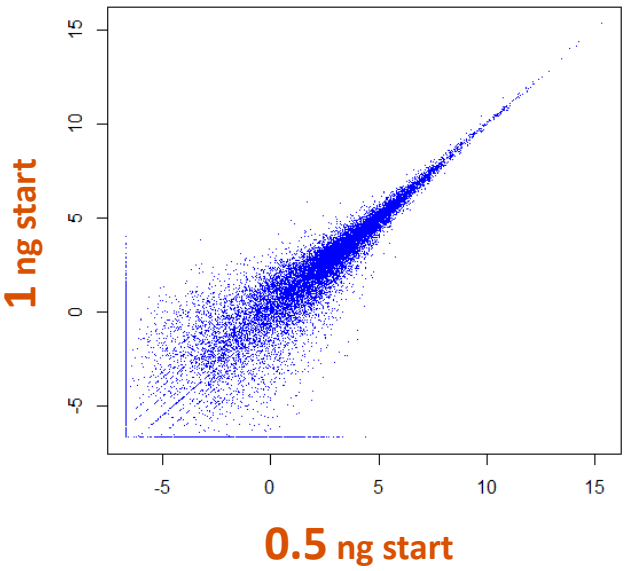



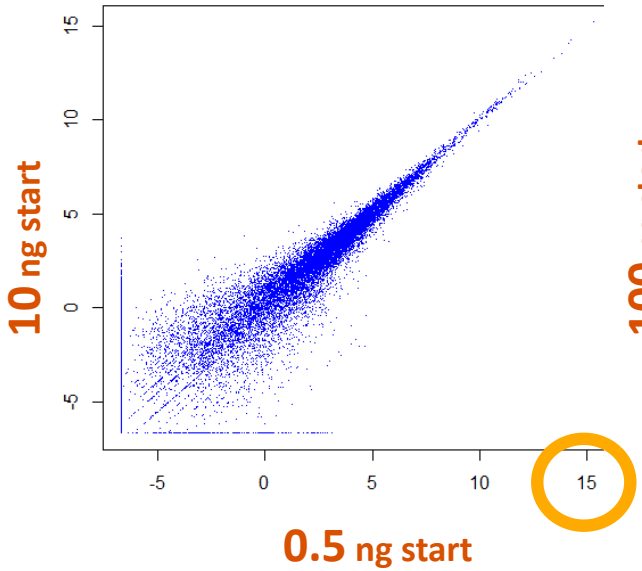

Cell line, 201B6
Microarray, 8X60K (Agilent)

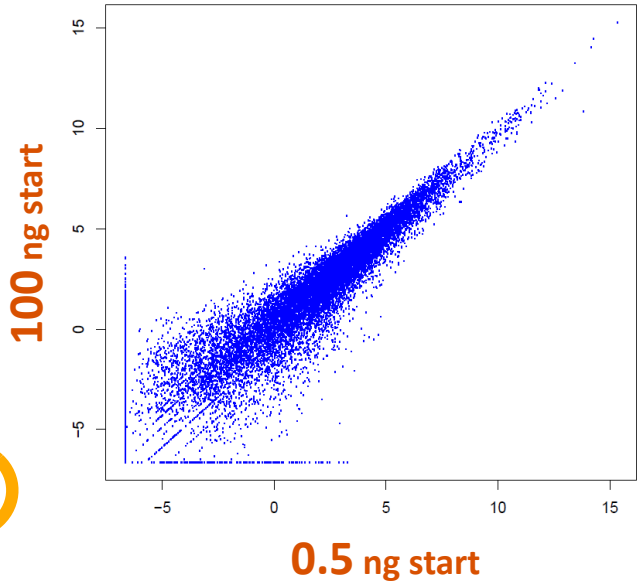
Yumi Inoue, Yuji Hashimoto & Naoki Amano

# Histogram of Transcription Level

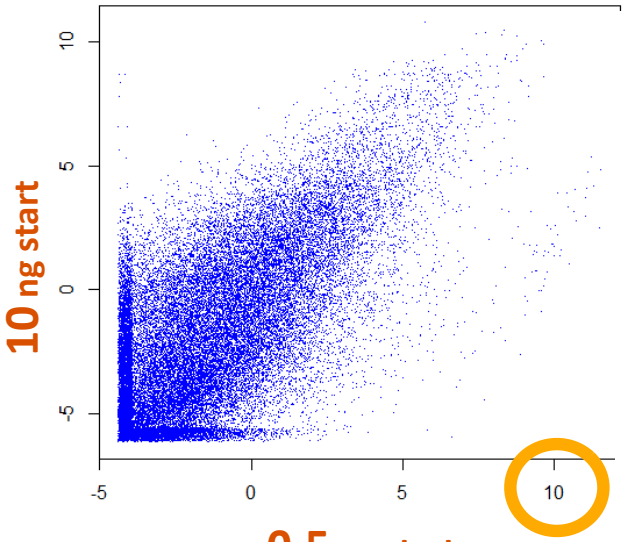
RNA-seq by Mondrian

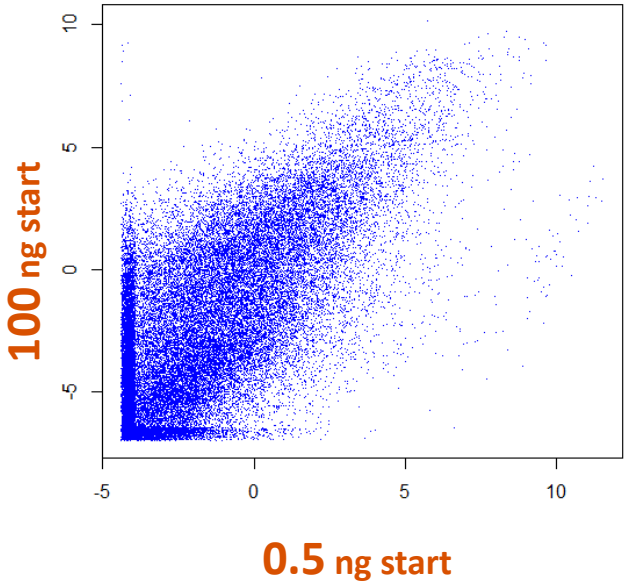



Microarray



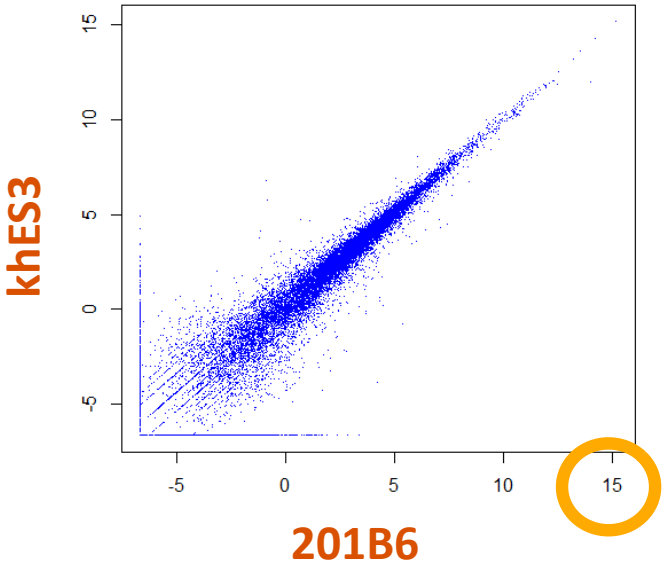

Cell line, 201B6

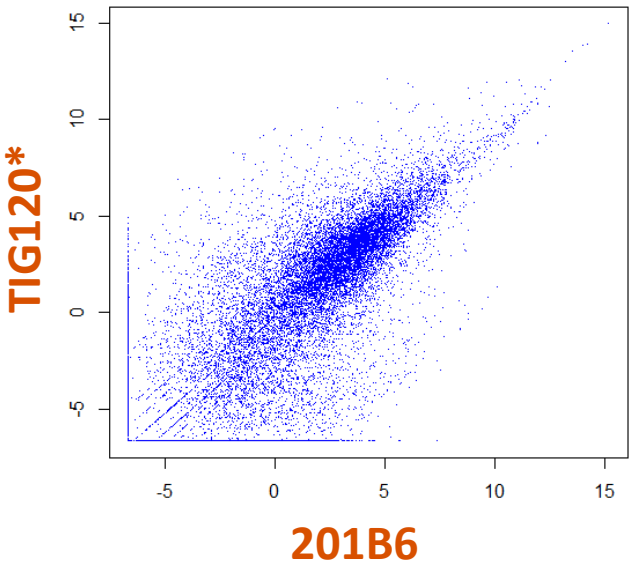
Microarray, 8X60K (Agilent)

Yumi Inoue, Yuji Hashimoto & Naoki Amano

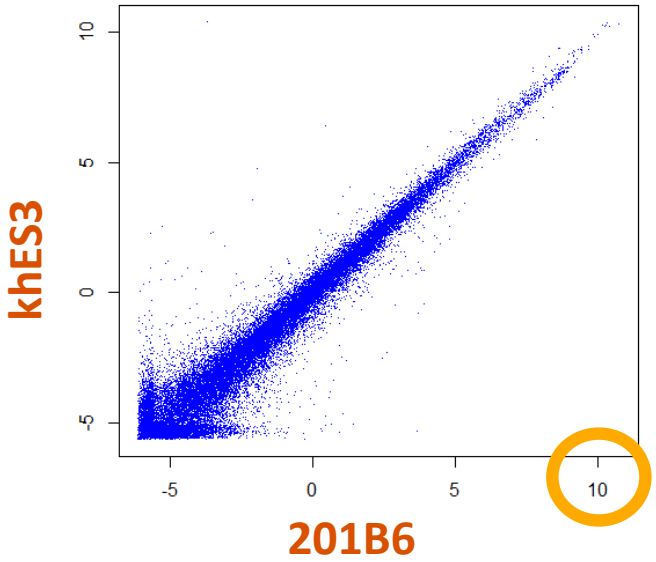
# High Dynamic Range

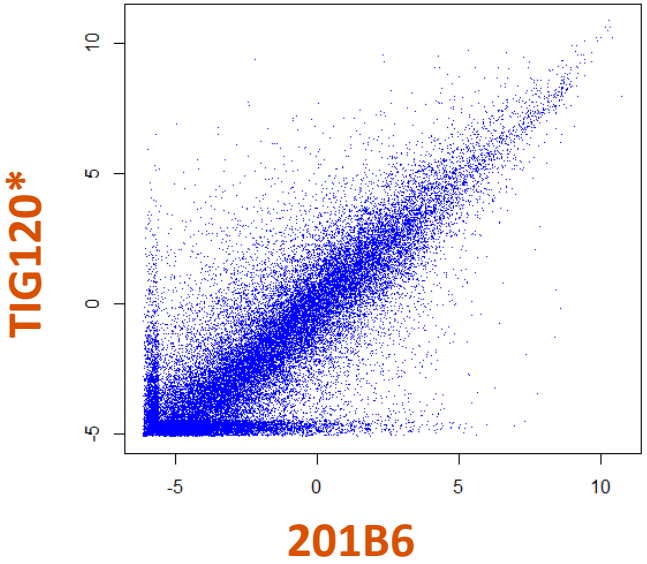
RNA-seq by Mondrian



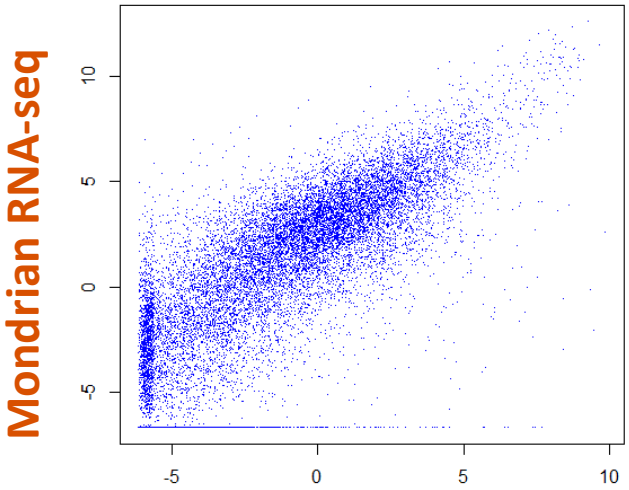

Microarray



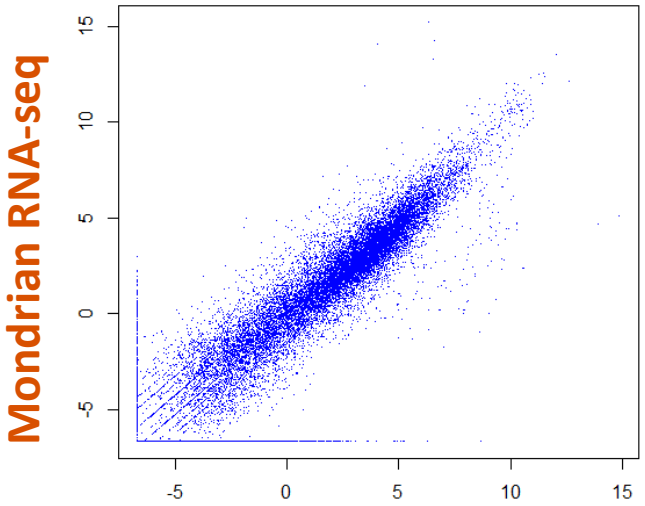




*HDF1388 was not available.

# Good Correlation: Different Reagent for RNA-seq



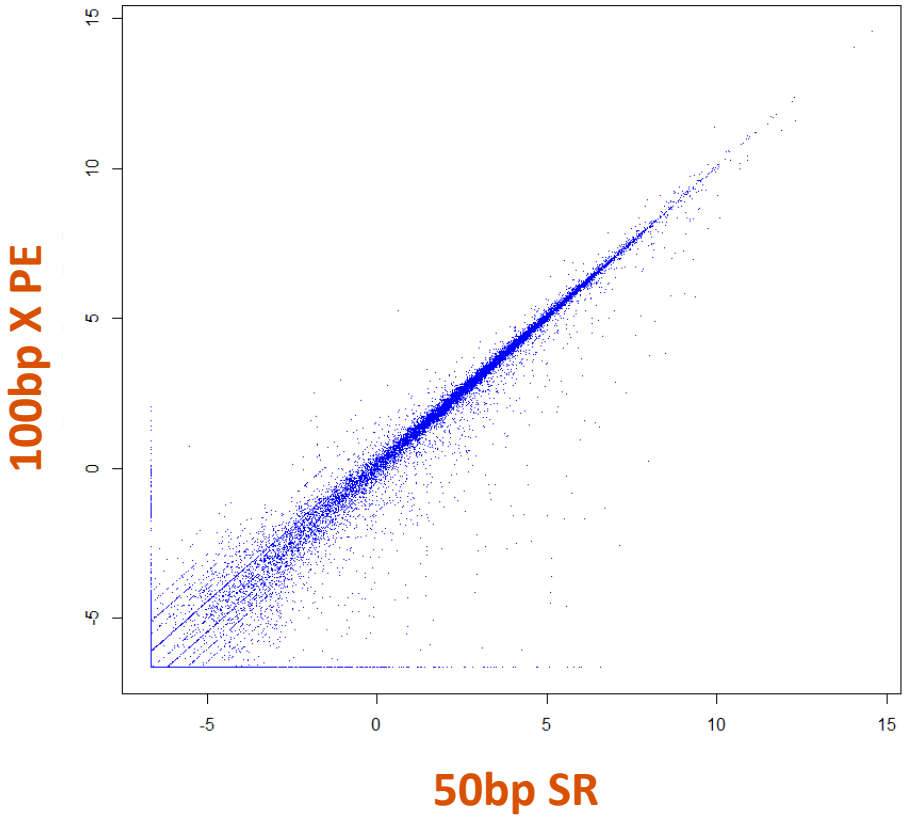

Microarray




TruSeq RNA-seq

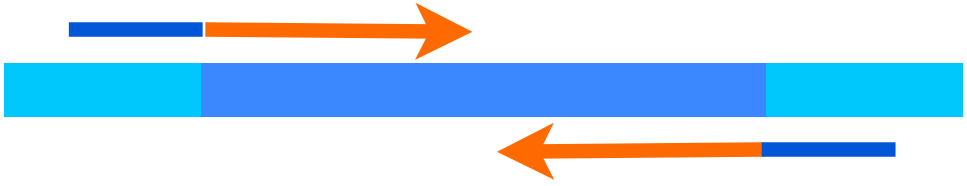
Cell line, 201B6

# Save the Cost and Time




201B6
50bp SR by TruSeq RNA-seq
100bp PE by TruSeq RNA-seq

100bp Paired end (total 200bp), **11** days



50bp Single Read, **3** days

なぜエピゲノムを調べるのか？

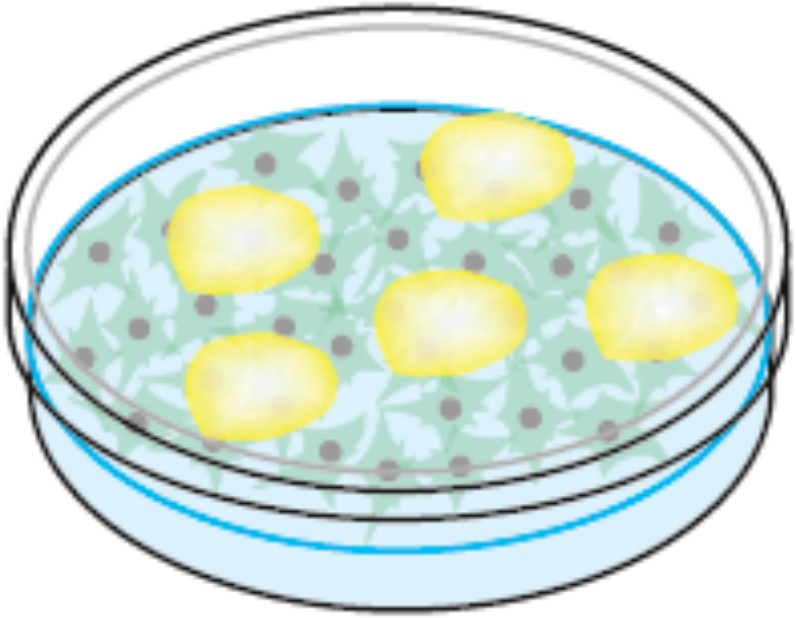
どう調べるのか？

# 次世代エピゲノム解析

pico-level transcriptome

single cell transcriptome

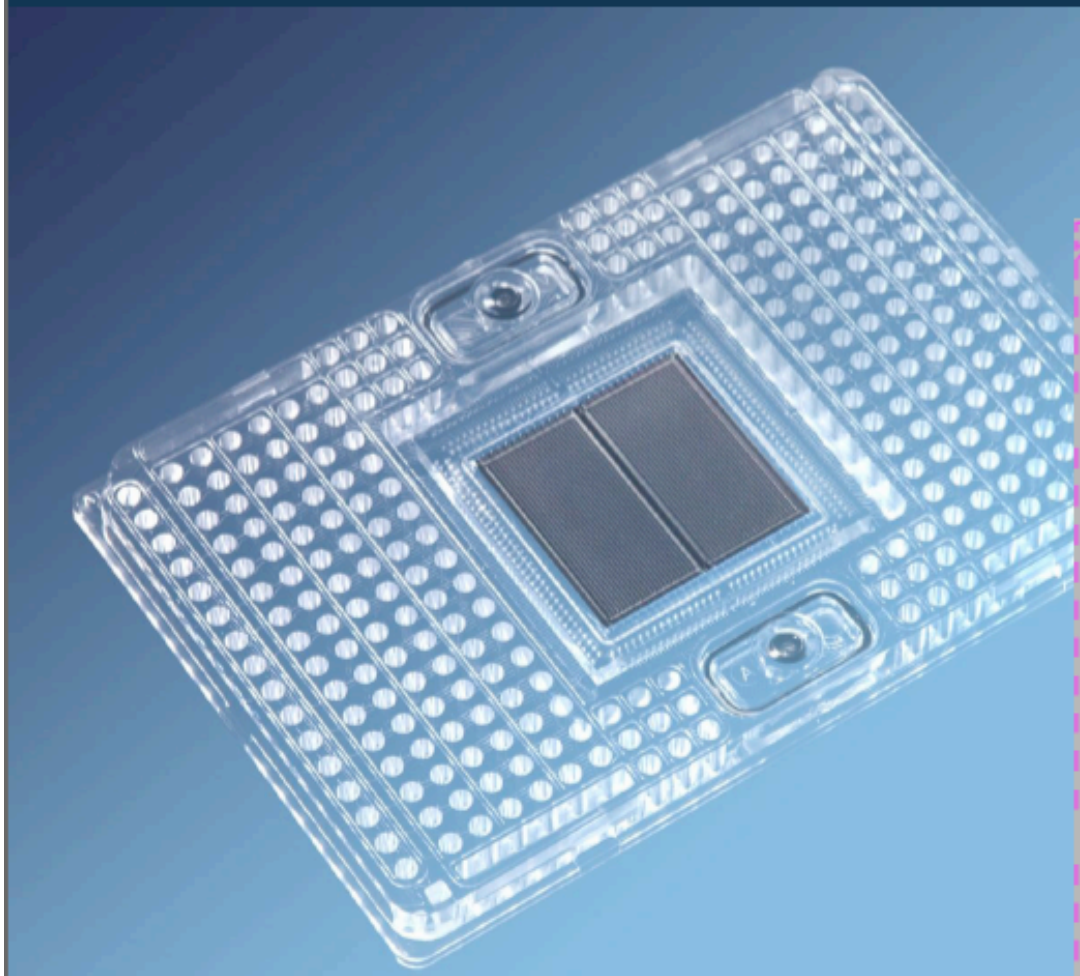
# Single Cell Analysis

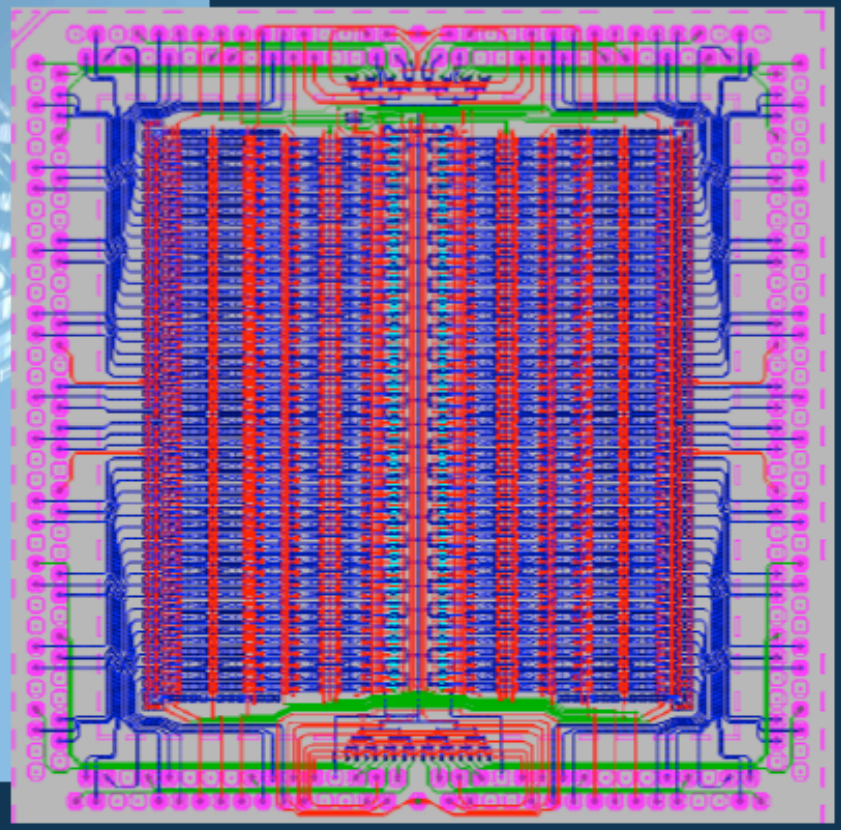


10^6 cells

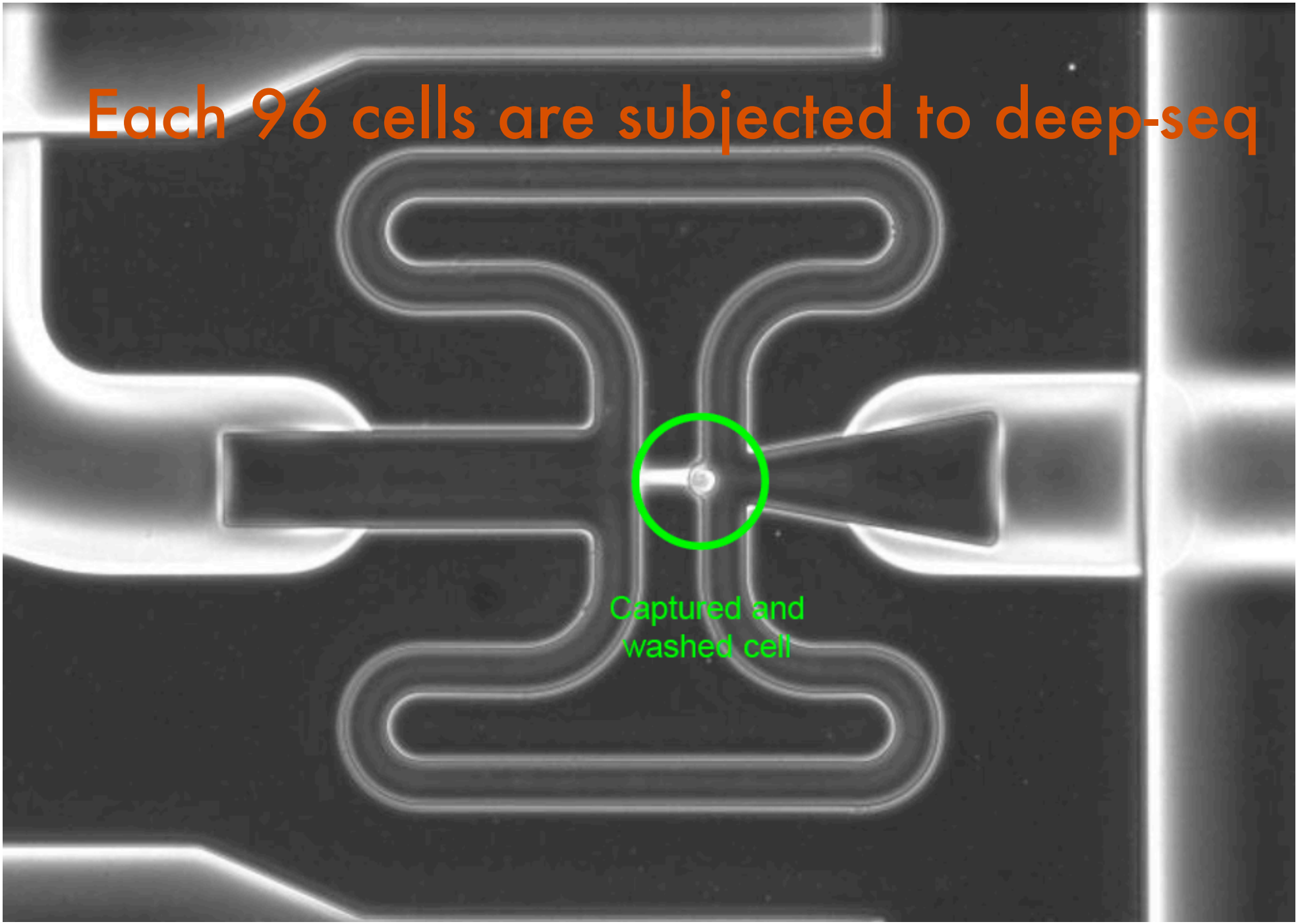

Specific Transcript Analysis
Whole Transcript Analysis
Whole Genome Analysis

# Single Cell Processing Architecture

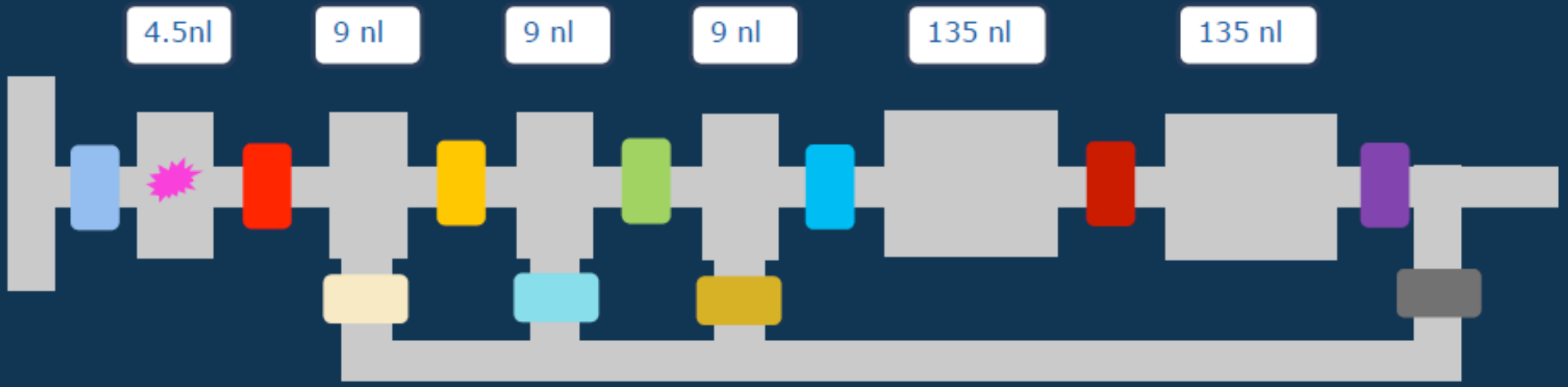


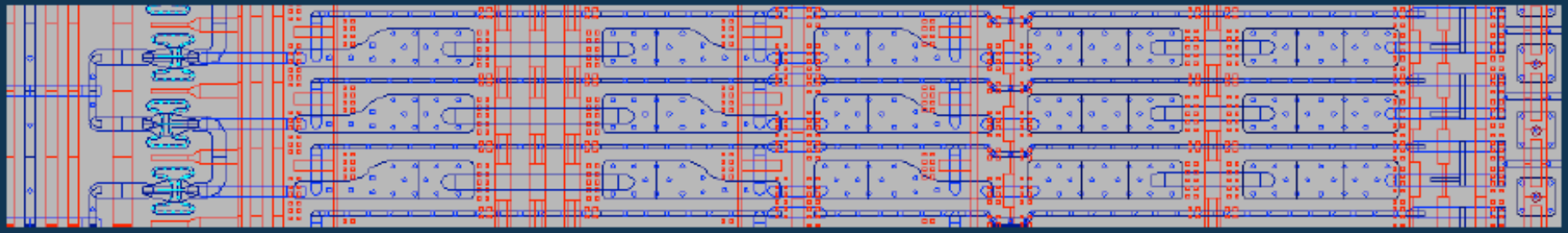


# Each 96 cells are subjected to deep-seq



# Multi-step reaction architecture

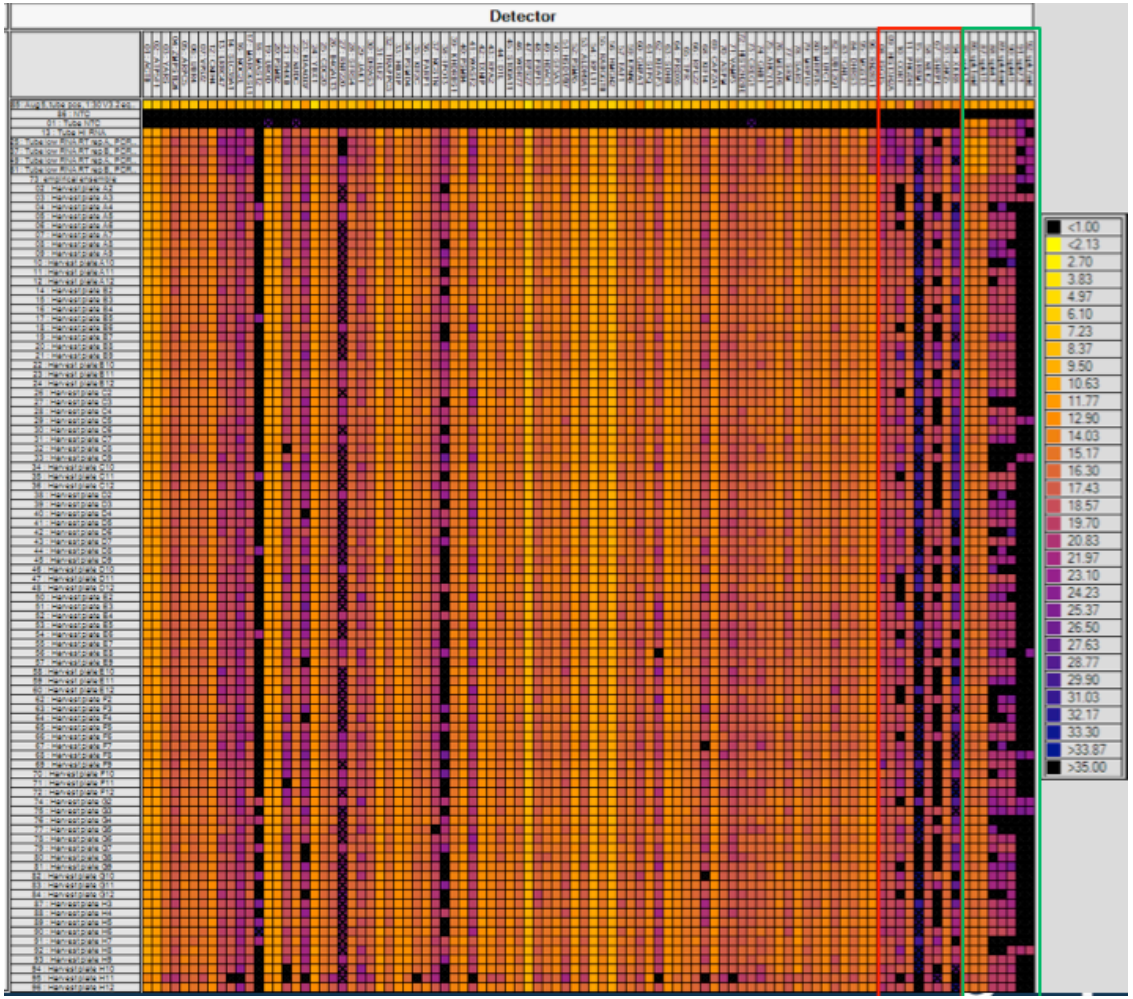


# High Reproducibility

96 targets

96 cells




Validated by BioMark

# HiSeq2000 x2

600Gb / 2weeks

Genome seq., exome, WGBS


# GAIIx

60Gb / 1weeks

ChIP-seq

# MiSeq

7Gb/27hrs

Amplicon seq.

SOLiD5500xL

454 FLX

## Server

12cores / 96Gb mem. X12

40cores / 1Tb X1

500Tb storage

# NGS現場の会

## 2013.9.4-5

## 神戸国際会議場

大会長　二階堂　愛（理化学研究所　CDB）
副大会長　渡辺　亮（京都大学 CiRA）

## Next Generation Society

NGSField

http://ngs-field.org/

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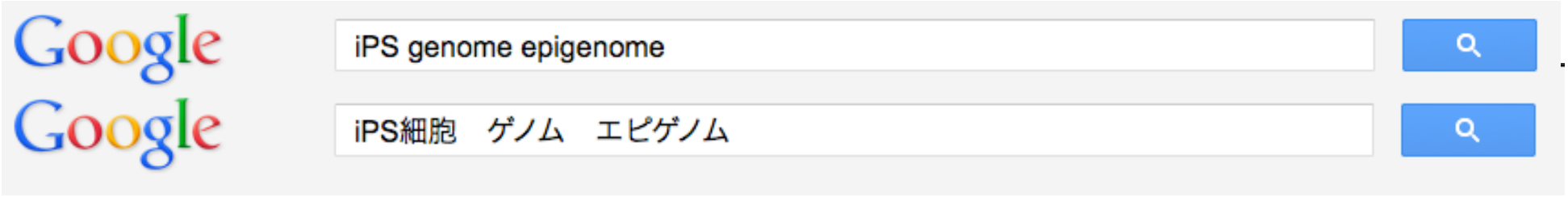
http://www.cira.kyoto-u.ac.jp/watanabe

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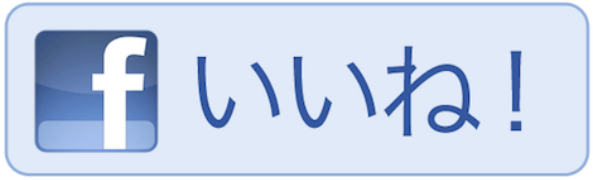


a.watanabe@cira.kyoto-u.ac.jp