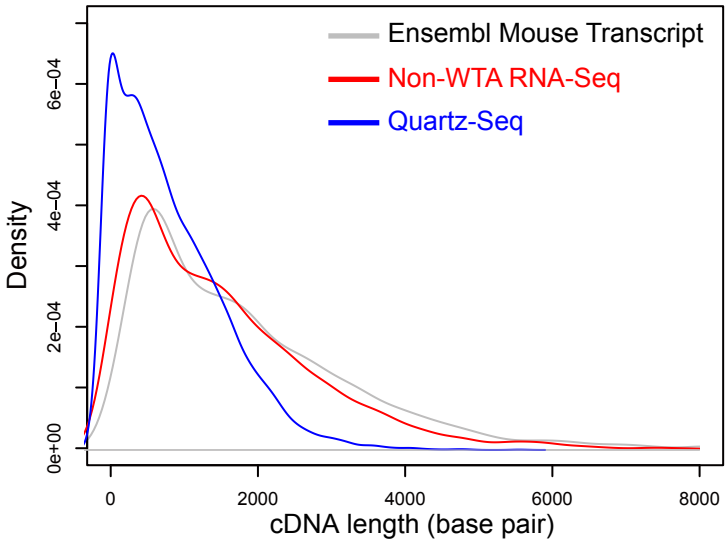
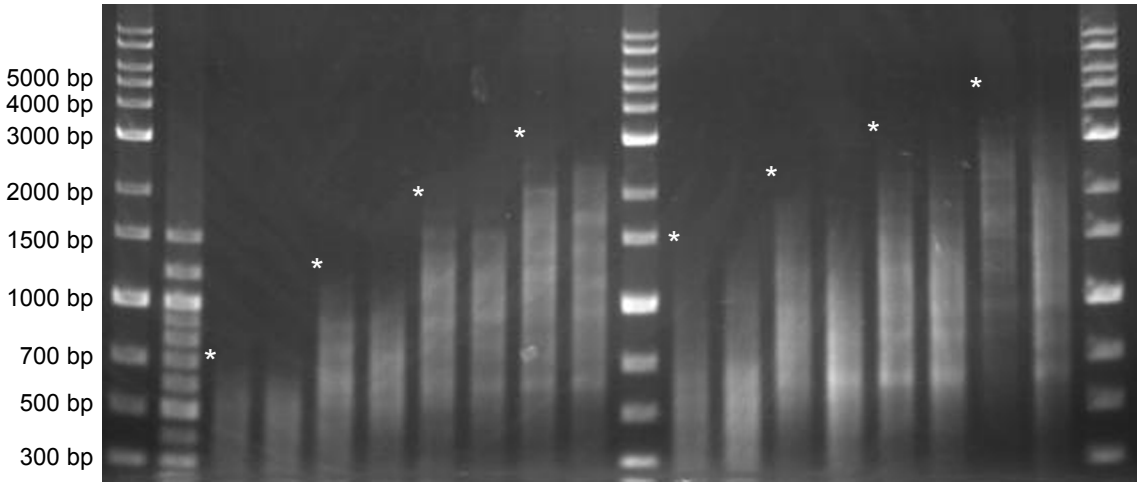
RTのコントロール
～cDNAの長さ1～

a

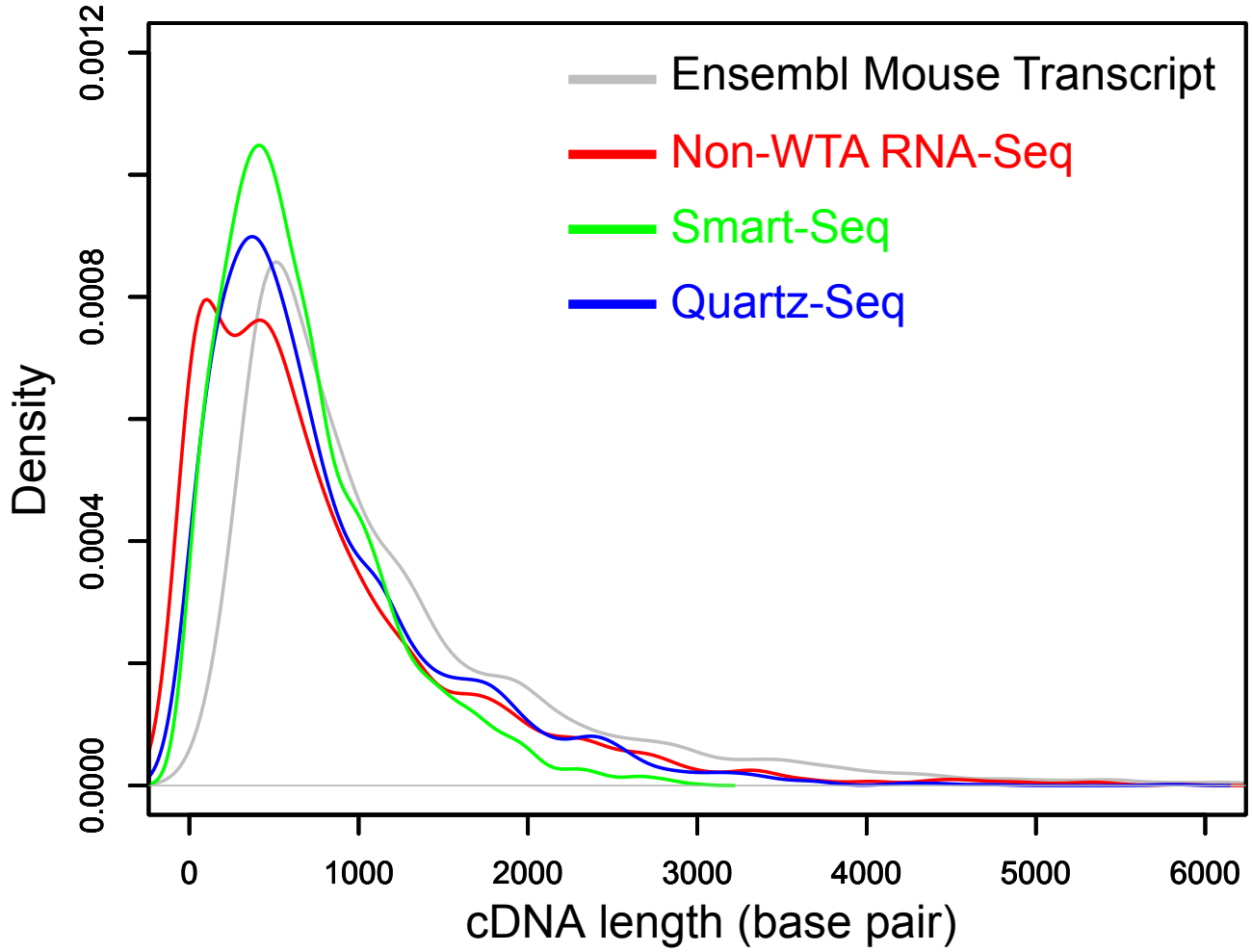


cDNAの長さが長くなり過ぎないよう
逆転写反応の時間と条件を決定。
3’ 側にかたよる。

b



RTのコントロール
～cDNAの長さ2～



一方でcDNAの長さで他の方法と遜色はない。
Smart-Seqで検出された遺伝子にあわせて解析。

Sasagawa *et al. Genome Biology* 2013, **14**:R31
http://genomebiology.com/2013/14/4/R31

Genome Biology

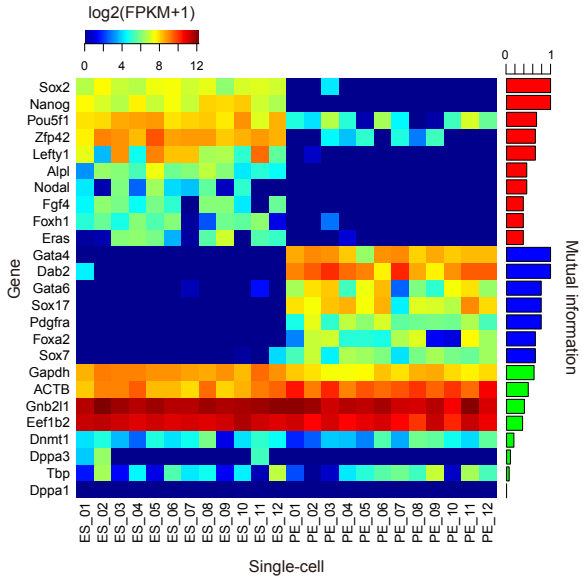
METHOD Open Access

Quartz-Seq: a highly reproducible and sensitive single-cell RNA sequencing method, reveals non-genetic gene-expression heterogeneity

Yohei Sasagawa[1,7†], Itoshi Nikaido[1,7†], Tetsutaro Hayashi[2], Hiroki Danno[3], Kenichiro D Uno[1], Takeshi Imai[4,5] and Hiroki R Ueda[1,3,6*]

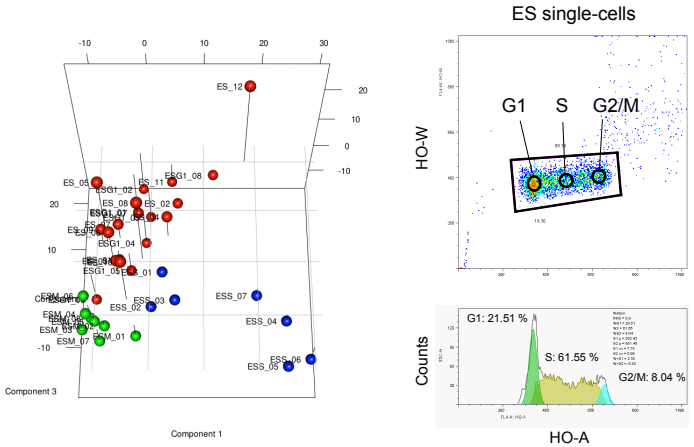
分化状態の違い

a



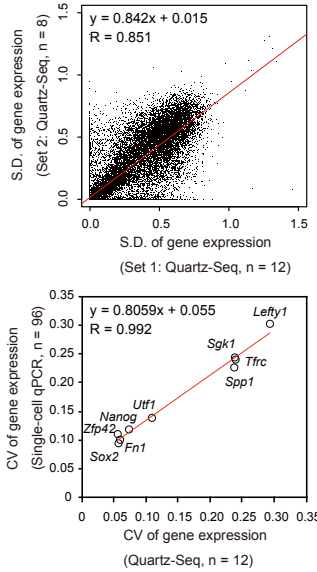
細胞周期の違い

b



遺伝子発現のばらつき

c



1細胞RNA-seqを行うにあたって

1．1細胞でやる意義・評価系・検証系（実験デザイン）

2．1細胞実験の環境コントロール

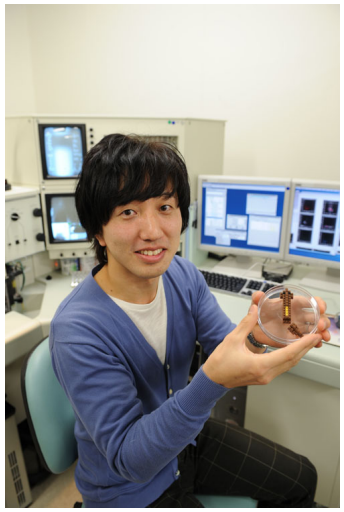
3．1細胞RNA-seq法（Quartz-Seq）の導入

4．1細胞採取方法の導入

5．アップデート

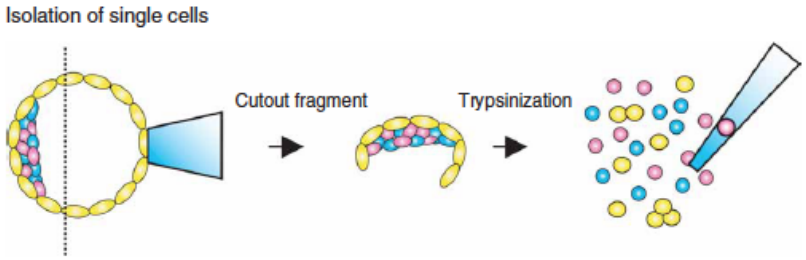
1細胞回収
～Quartz-Seqの場合～

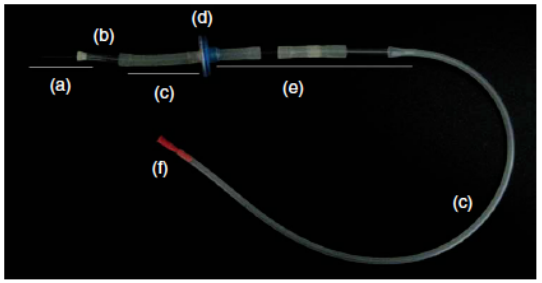
浮游細胞の回収
～Cell sorterを用いた回収～



Tetsutaro HAYASHI
RIKEN CDB GRAS

浮游細胞の回収
～マウスピペットによる吸引～

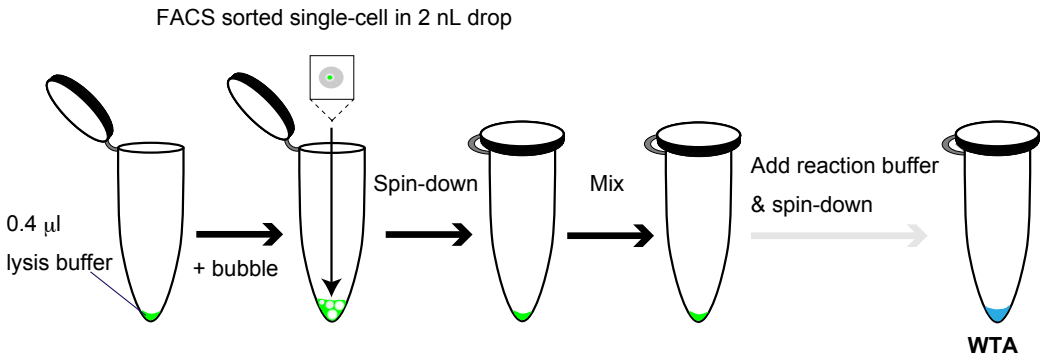


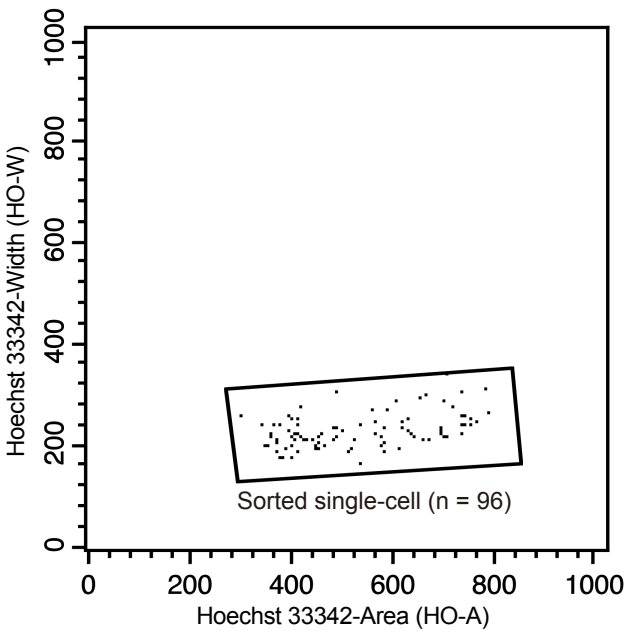


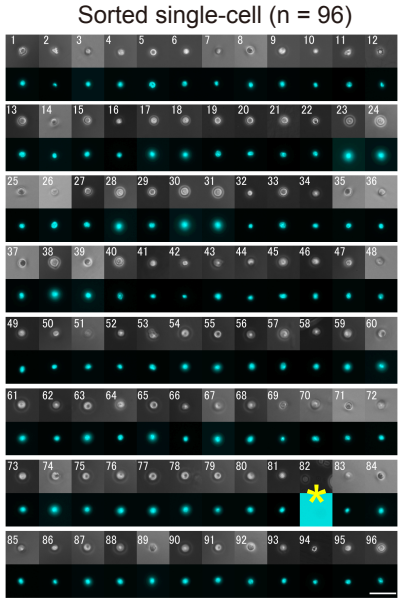
Nat Protocols 2007 2(3) 740, Kurimoto et al.

1細胞回収
～Quartz-Seqの場合～

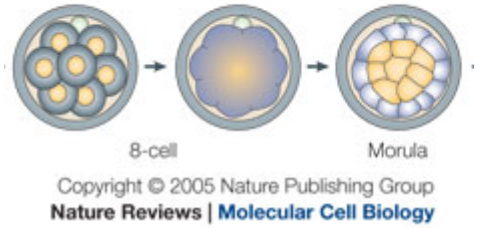
浮游細胞の回収
～Cell sorterを用いた回収～



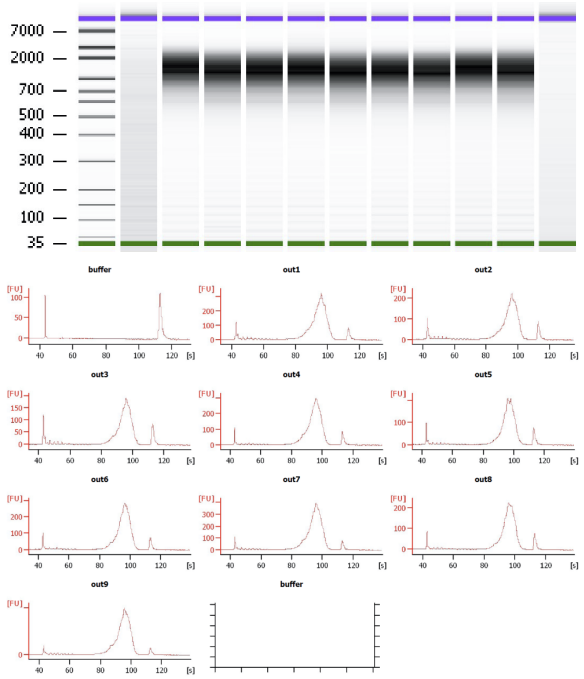




浮游細胞の回収
～マウスピペットによる吸引～

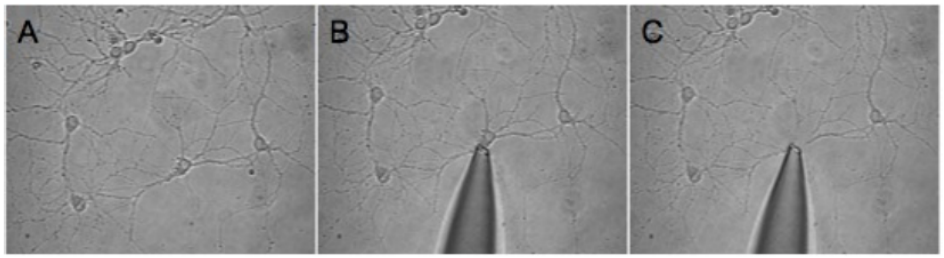


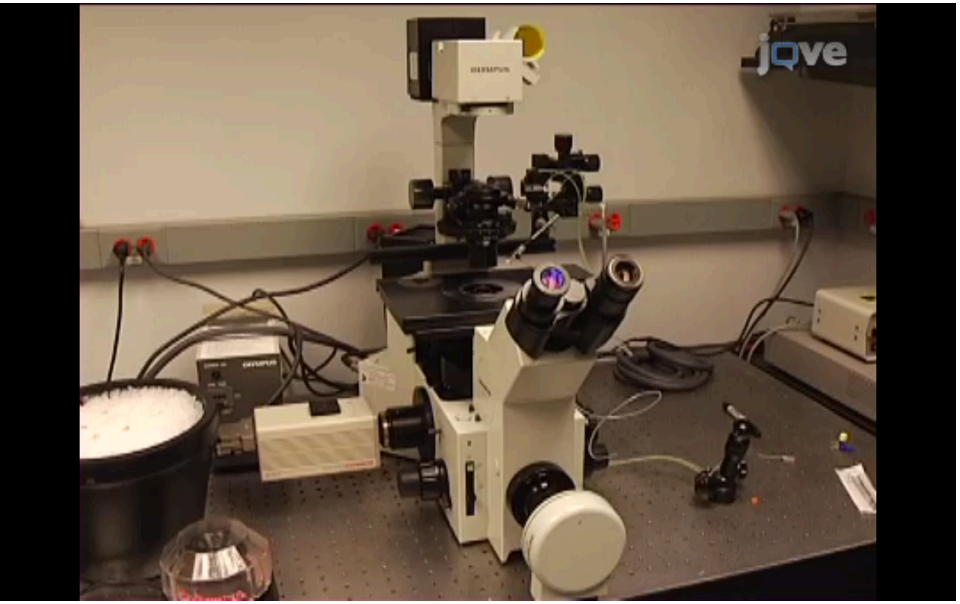
Quartzによる割球からの増幅cDNA



回収方法 その2

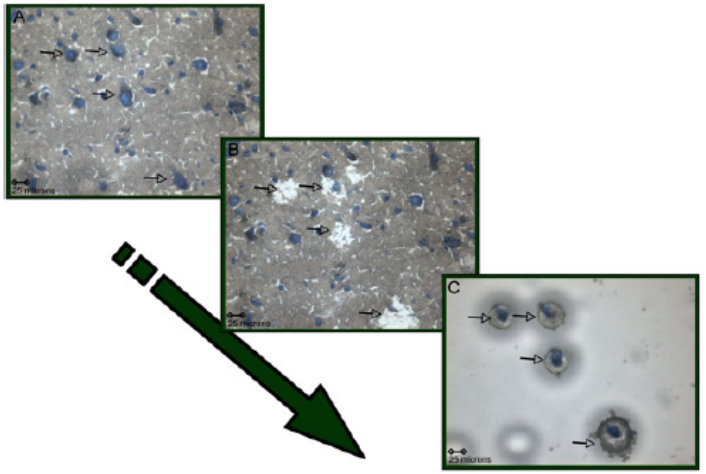
張り付いた細胞を回収 ***
～ガラスピペットによる吸引～





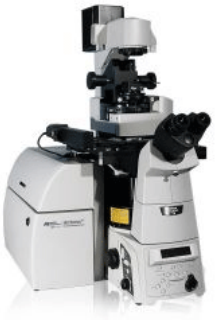
J Vis Exp. 2011 25(50) 2634, Morris et al.

スライスから回収 ***
～Laser Capture Microdissection (LCM)～



J Vis Exp. 2009 6(30) 1444, Pietersen et al.

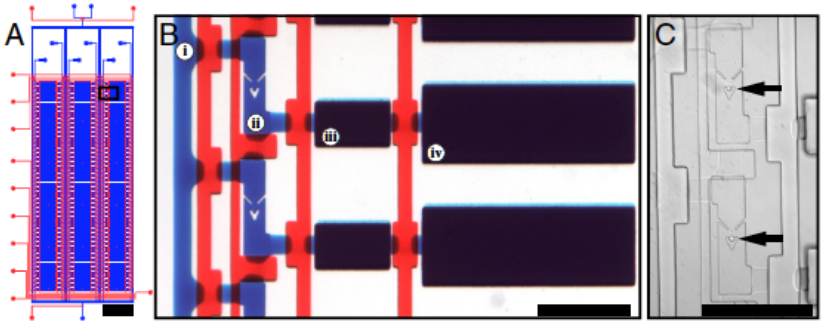
http://www.digital-biology.co.jp/allianced/products/cellcutplus/



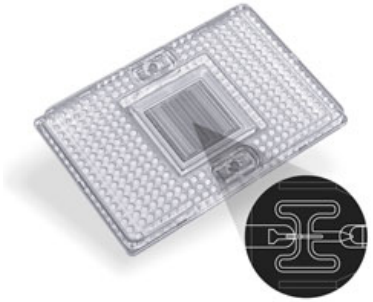
http://www.appliedbiosystems.jp/website/jp/product/modelpage.jsp?BUCD=139214&PLCD=139215&MODELCD=139216

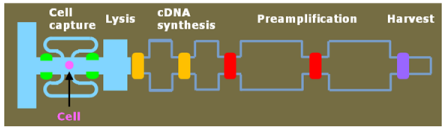
回収方法 その3

浮游細胞の回収
～微細流路の使用～



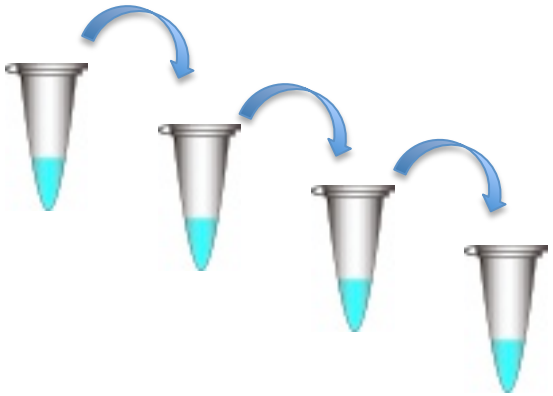
PNAS 2011 108(34) 13999, White et al.





http://www.fluidigm.com/c1-single-cell-auto-prep-system.html

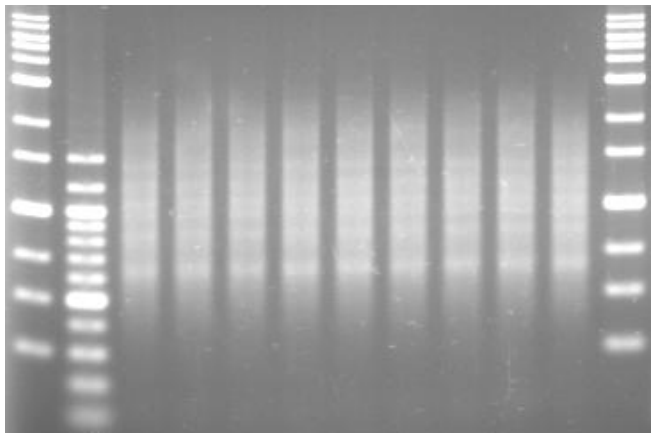
浮游細胞の回収
～限界希釈+ピペット～



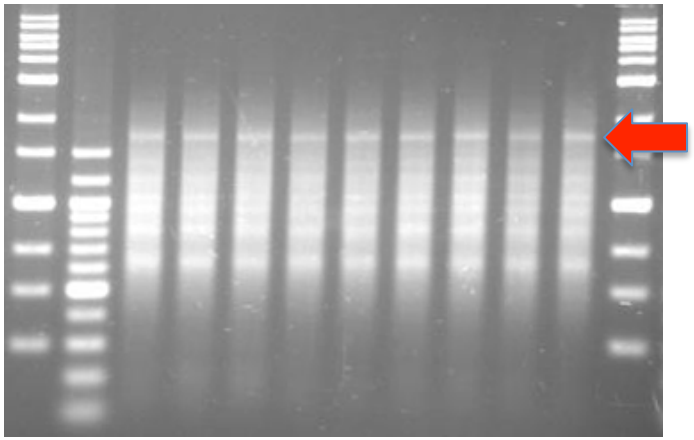
細胞の溶解条件

<界面活性剤の効果>

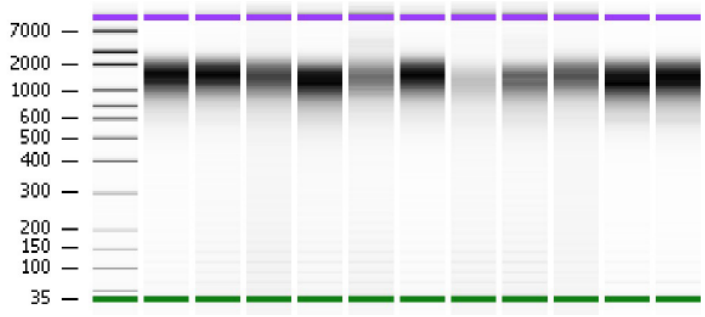
H_2Oによる浸透圧だけで破砕

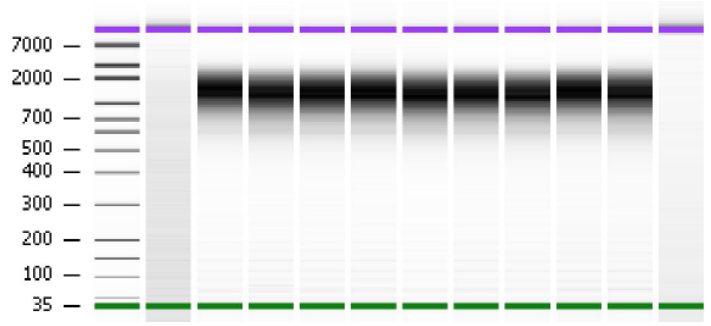


界面活性剤NP-40で破砕



<界面活性剤の濃度・時間の最適化>





1細胞RNA-seqを行うにあたって

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2．1細胞実験の環境コントロール

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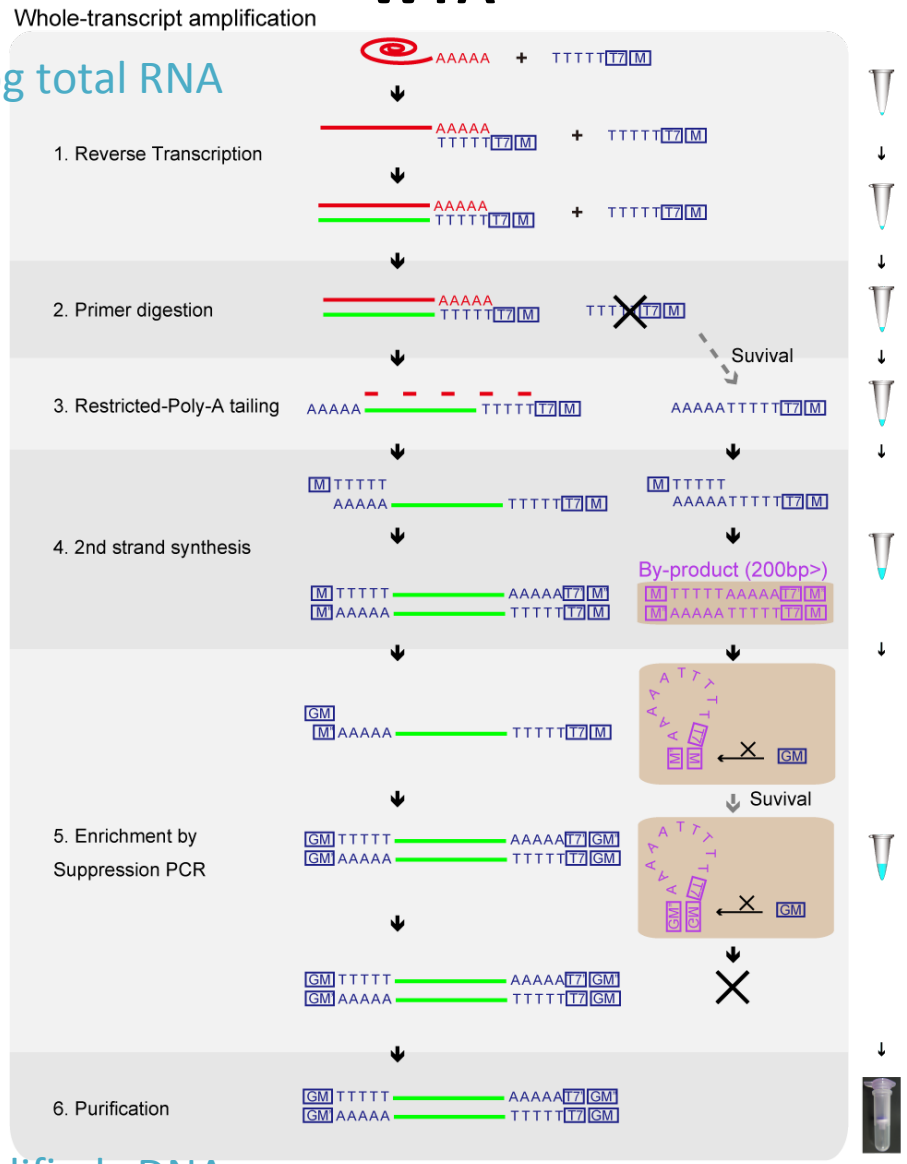
4．1細胞採取方法の導入

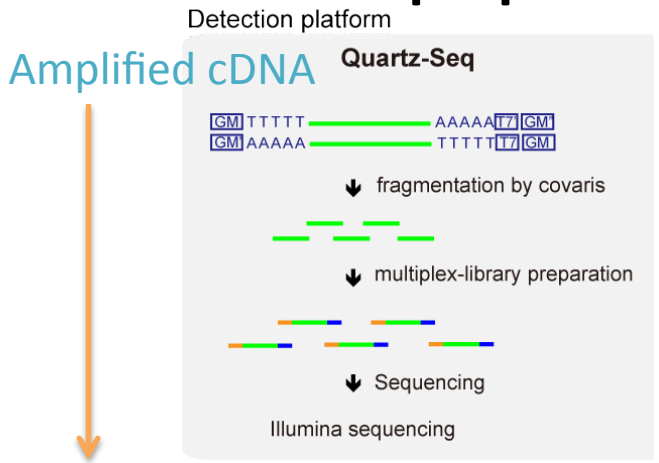
5．アップデート

Quartz-Seq法の概要

WTA



LIMprep



LIMprep

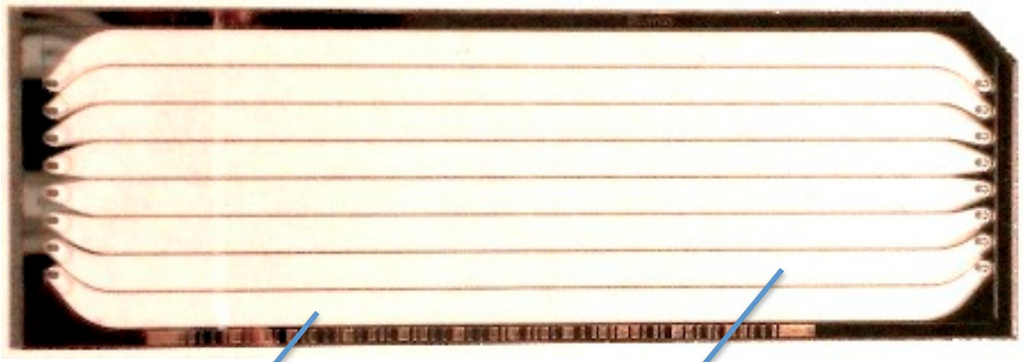
3-fold efficiency

HALprep

5-10 ng DNAから
非増幅ライブラリ作製可能

シーケンスライブラリDNAの必要量 1

http://www.illumina.com/



Singleplex (single / lane)
Library DNA (length 321 bp)
235 pg / sample
2 nM 0.6 ul (10 pM 120 ul / lane)

Multiplex (9-plex / lane)
Library DNA (length 321 bp)
26 pg / sample
2 nM 0.6 ul (10 pM 120 ul / lane)

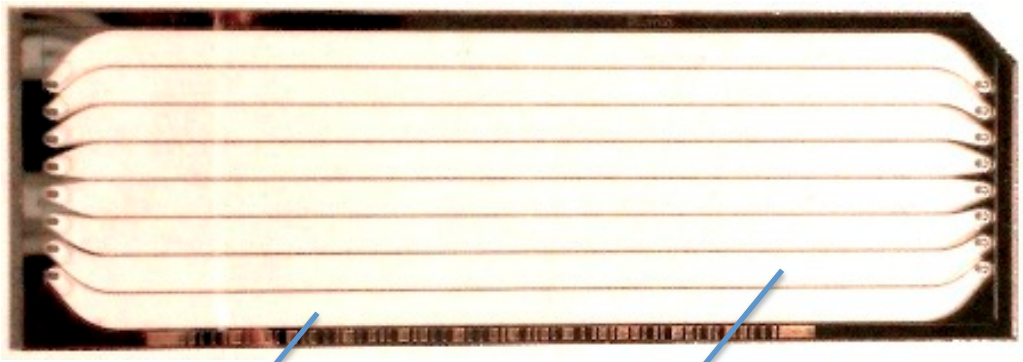
シーケンスライブラリDNAの必要量 2

Adaptor ligation 100%

Start material: 146 pg / sample
Start material: 16pg / sample

→

LD: 235 pg / sample
LD: 26 pg /sample



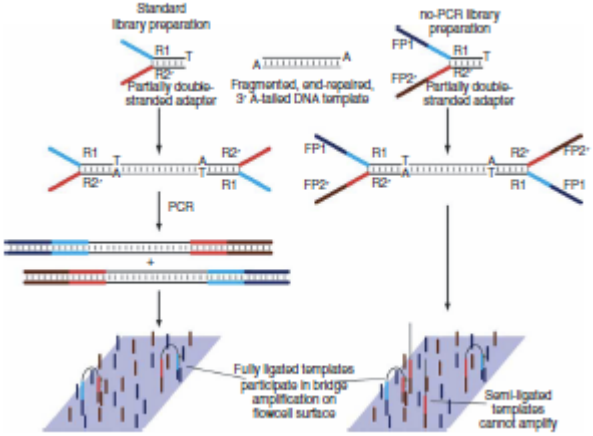
Singleplex (single / lane)
Library DNA (length 321 bp)
235 pg / sample
2 nM 0.6 ul (10 pM 120 ul / lane)

Multiplex (9-plex / lane)
Library DNA (length 321 bp)
26 pg / sample
2 nM 0.6 ul (10 pM 120 ul / lane)

Previous method: 1 ug genome DNA

Amplification-free Illumina sequencing-library preparation facilitates improved mapping and assembly of (G+C)-biased genomes

Iwanka Kozarewa[1,2], Zemin Ning[1,2], Michael A Quail[1], Mandy J Sanders[1], Matthew Berriman[1] & Daniel J Turner[1]



Nature methods 2009

LIMprep

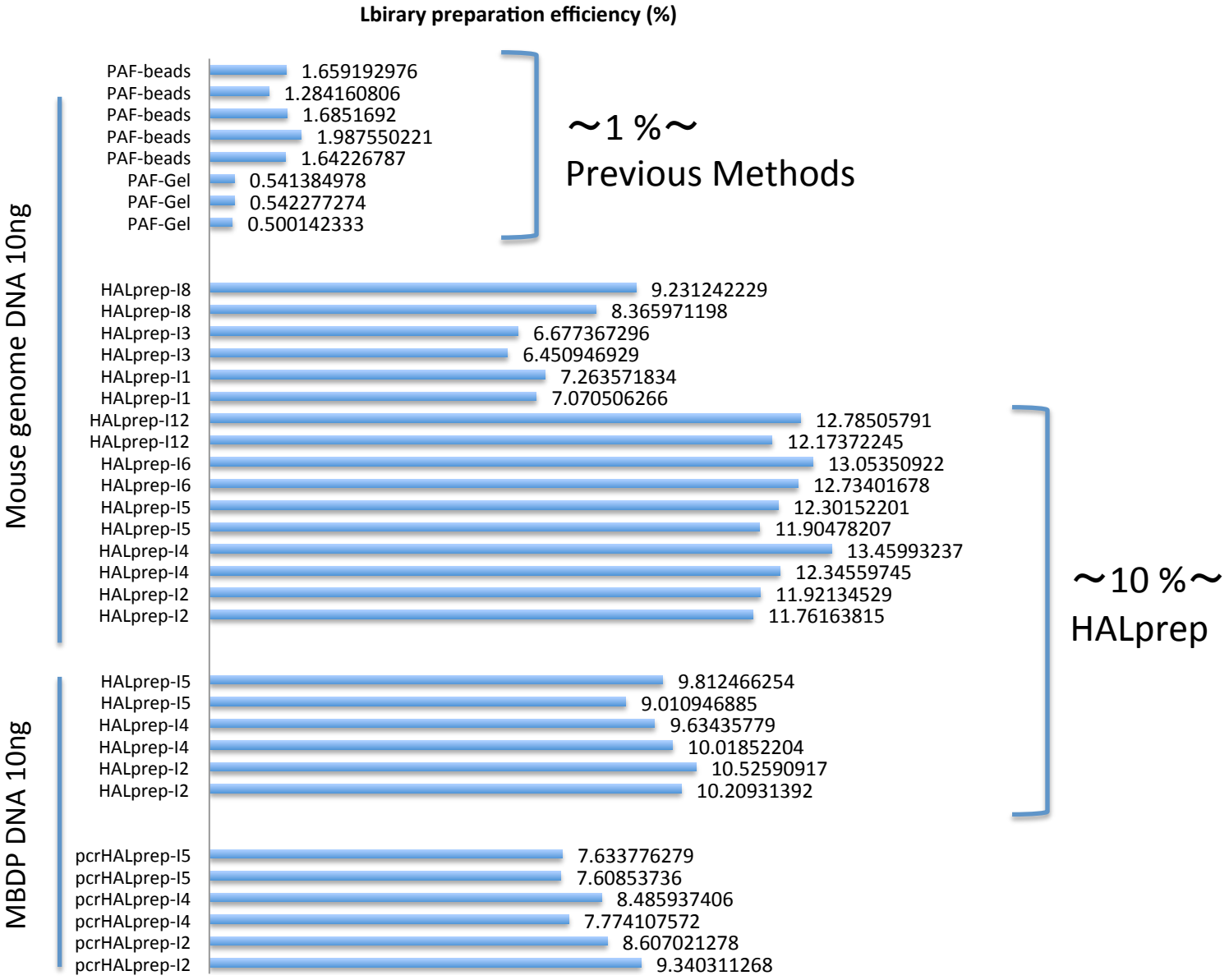
3-fold efficiency

HALprep:

a High-sensitive Amplification-free Library PREParation method

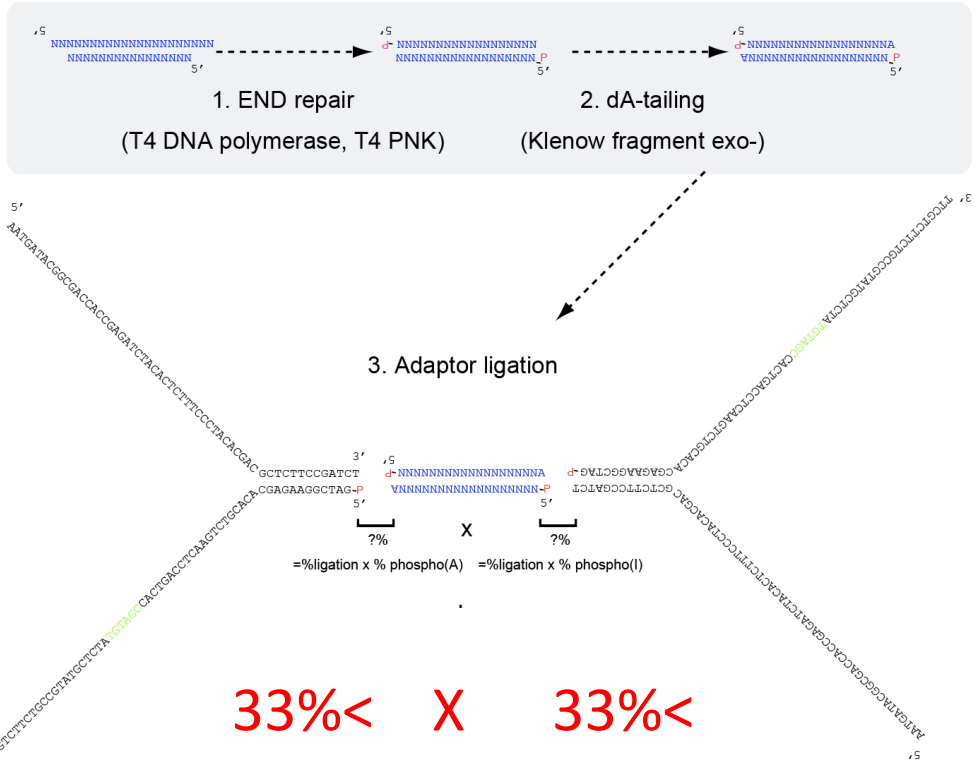
5-10 ng DNA

シーケンスライブラリDNAへの変換効率



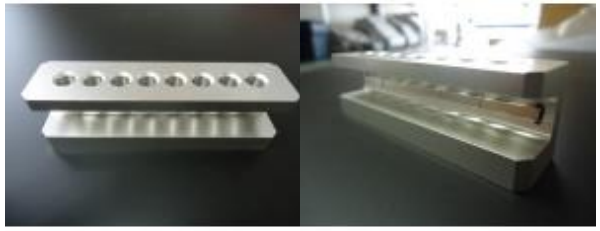
アダプターライゲーション効率は33%以上

1. **Adaptor**
2. **DNA Purification**
3. **Enzyme condition**

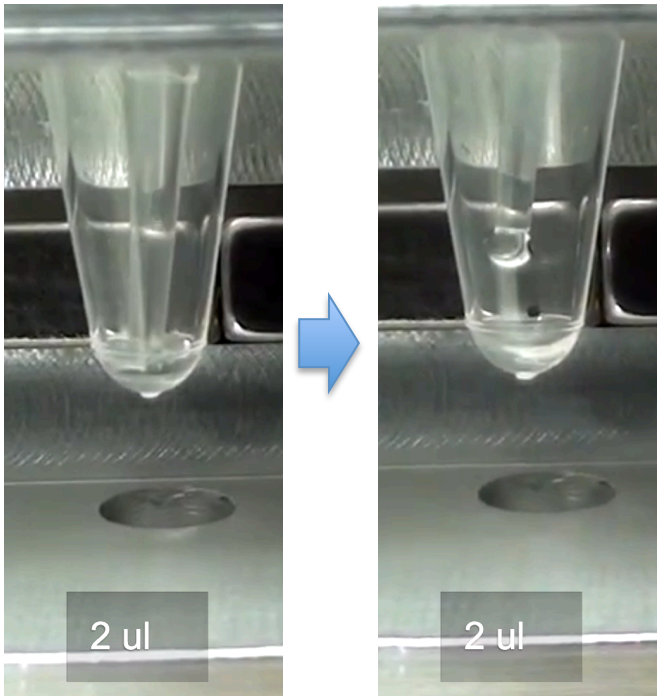


日本ジェネティクス角川さんと
一緒に商品化しました。

http://www.n-genetics.com/product_detail.html?item_id=4608



2 μL程度のサンプルを精製可能



HALprepのワークフロー

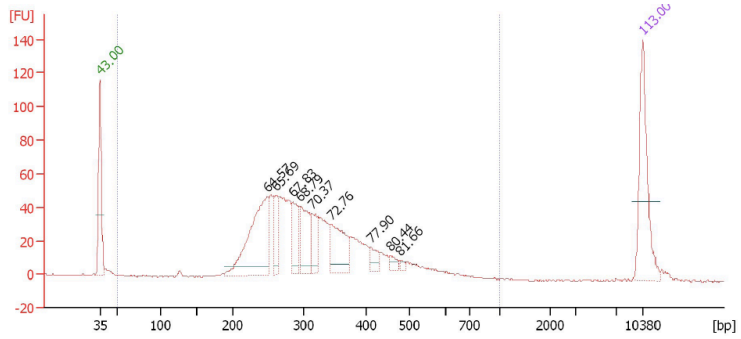
End repair

dA-tail

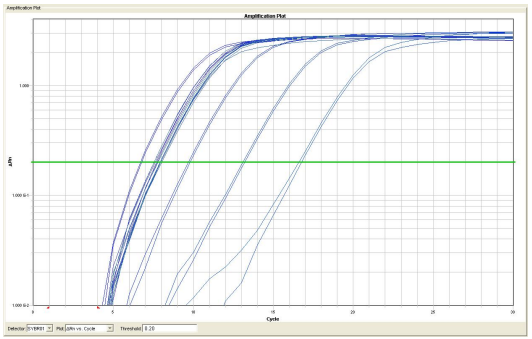
Adaptor ligation

5-10ng (1 sample / 1 lane)
1-2ng (5 sample / 1 lane)

Check DNA Size after PCR
5% adaptor ligated DNA



Check DNA amount
5% adaptor ligated DNA

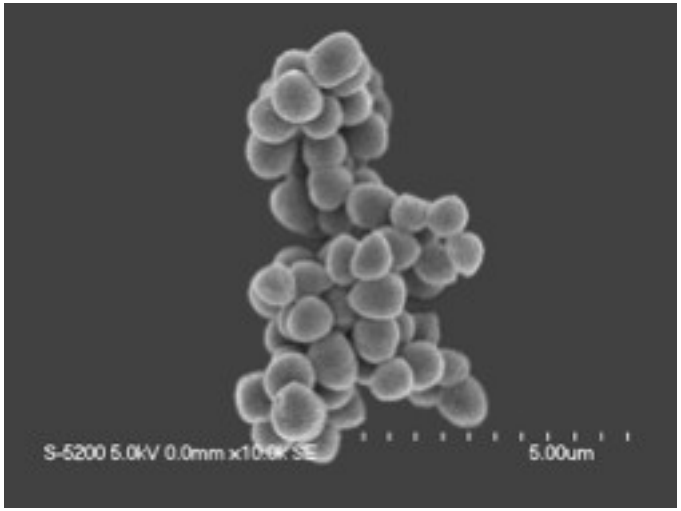


KAPA library quantification kit

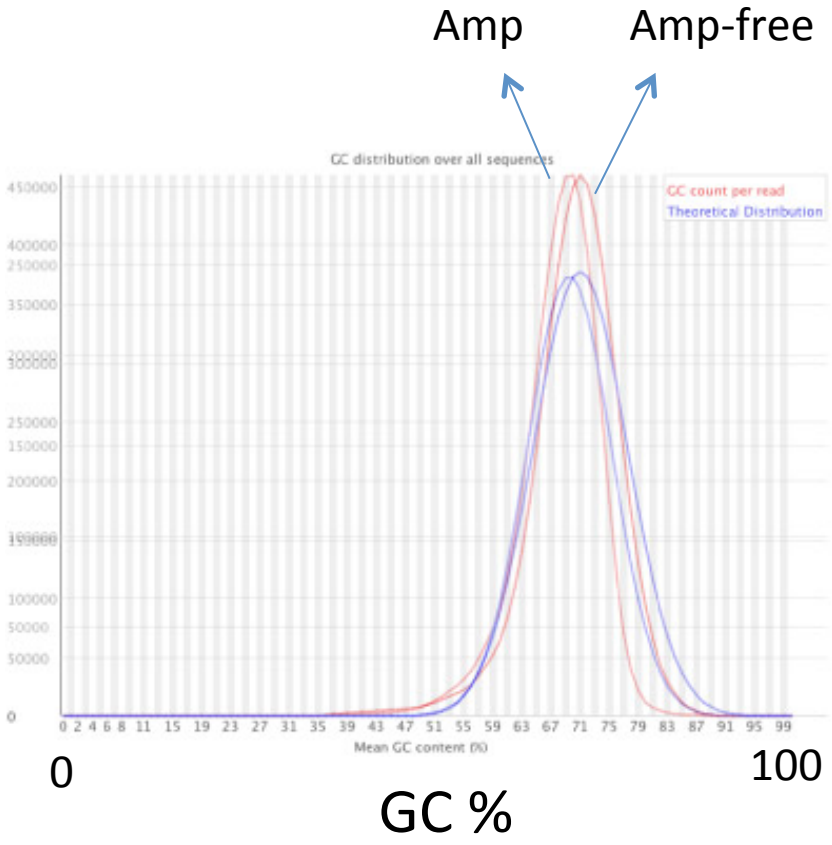
For Sequence
Rest DNA

*K. rhizophila*ゲノムDNAを用いたシーケンス

K. rhizophila
2.7M base, GC70%



Original data was downloaded from under web site.
http://www.bio.nite.go.jp/ngac/DC2201.html Kocuria rhizophila 電子顕微鏡写真
田村（NITE　NBRC）撮影

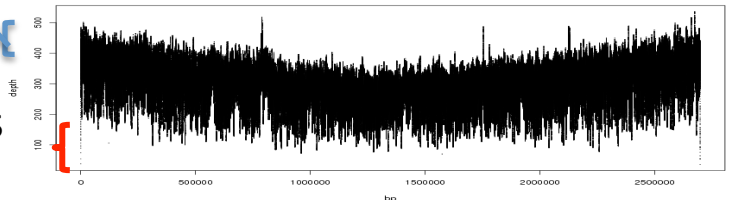


*K. rhizophila*ゲノムDNAを用いたシーケンス

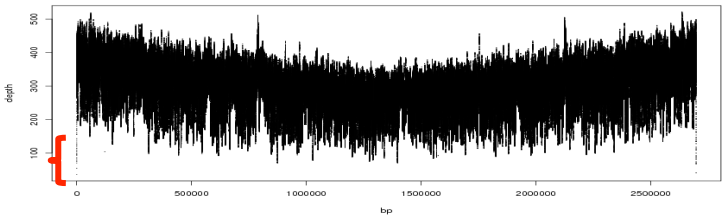
100x depth

Amp-free: HALprep n=2 ,10 ng

4M reads

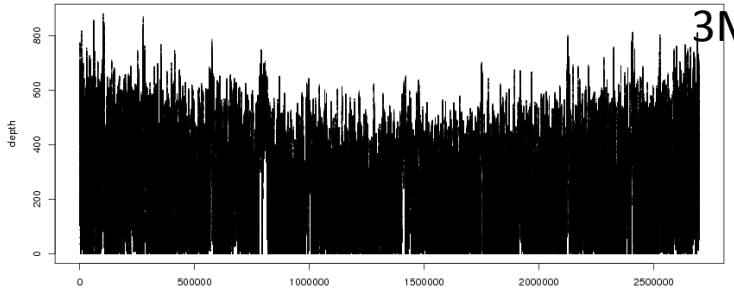


4M reads

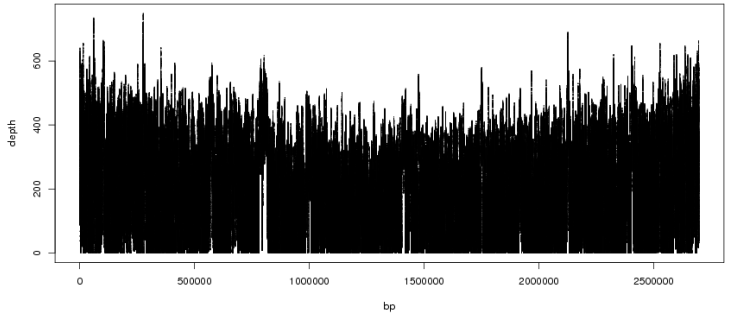


Amp: 10 cycle PCR with TruSeq DNA, 10 ng

3M reads



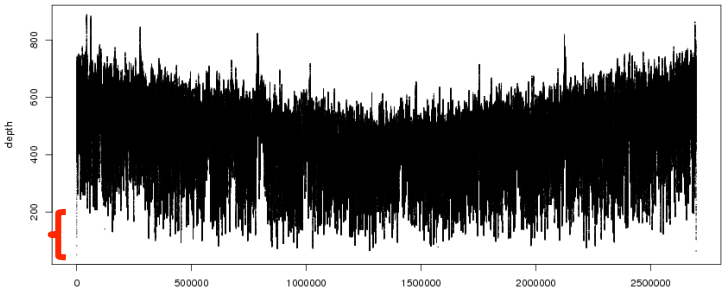
3M reads



Bias!!!

Amp-free: Previous method n=1 ,100 ng

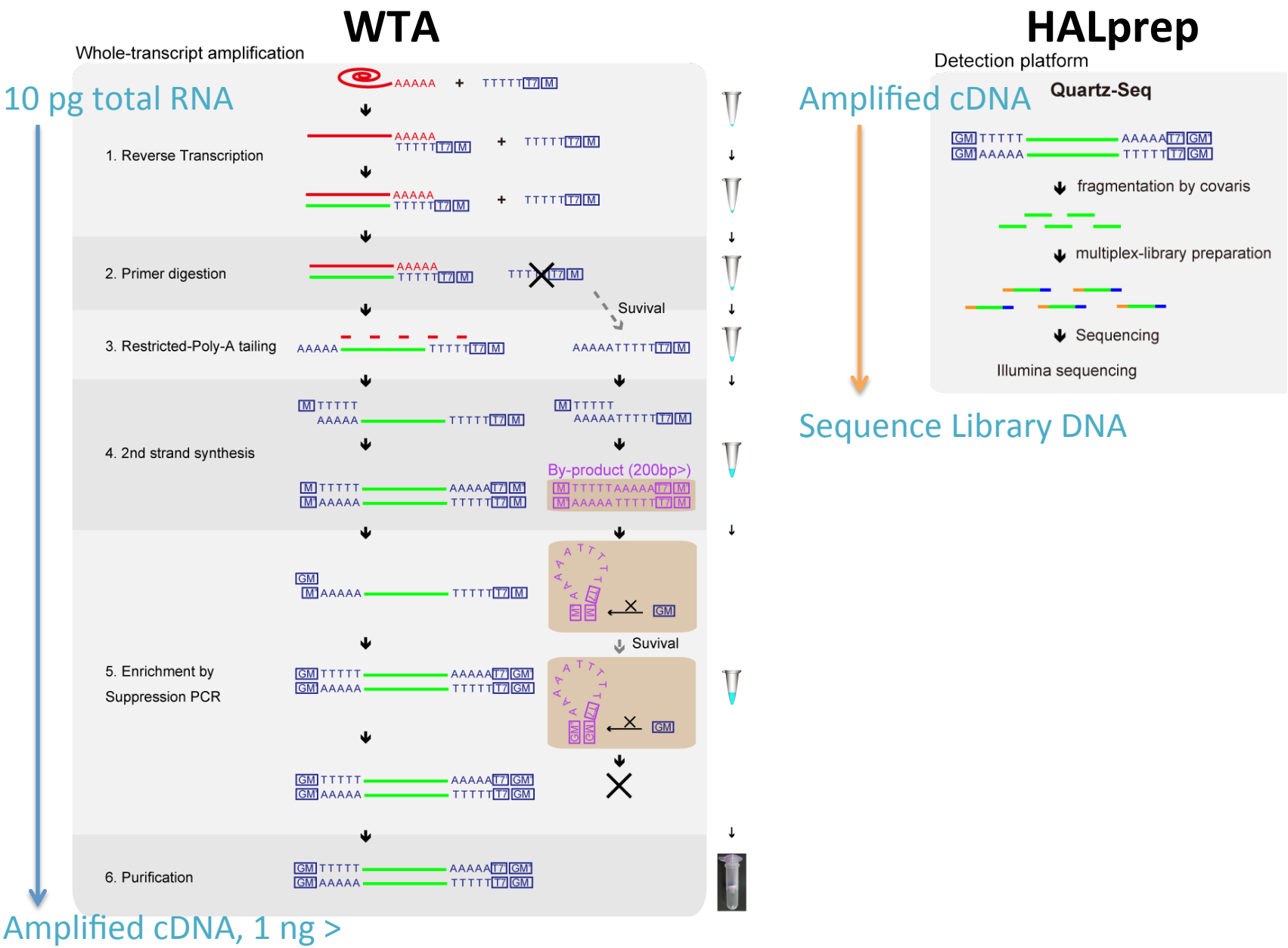
6M reads



0 2.7M

MiSeq v1 kit : PE 101bp

Quartz-Seq法+の概要



今後の展開

～1 細胞 RNA-Seq～

1. 定量性

   Quartz-Seq 以上

2. スループット

   数十レベルから数万レベルへ

3. ターゲット RNA

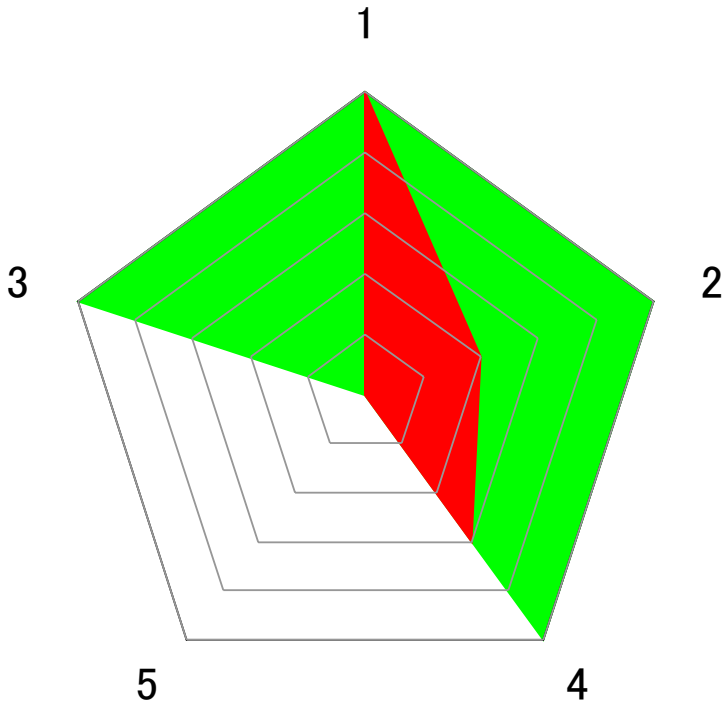
   non-poly-A RNA

4. トランスクリプトの構造

   長さ , 方向性

5. 別のレイヤーの同時測定

   genome, epigenetics (methylation etc)

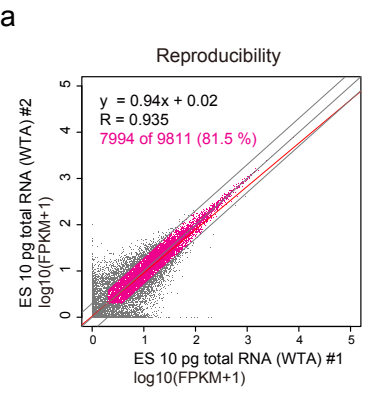


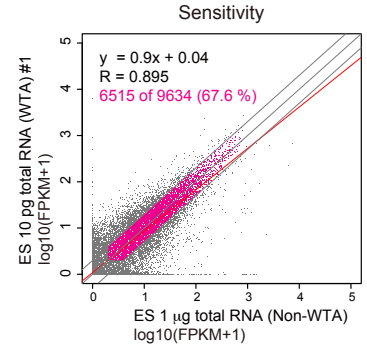
赤：Quartz-Seq、 緑：目指すべき開発の方向性

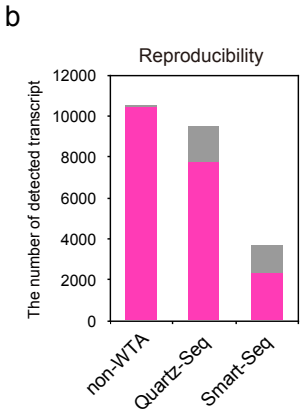
ガイドラインを参考にがんばってください。

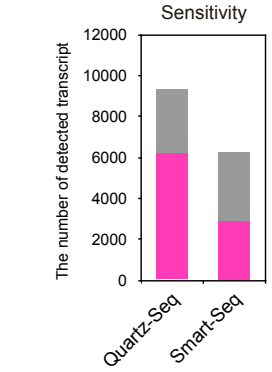
ご清聴ありがとうございました。

補足スライド1

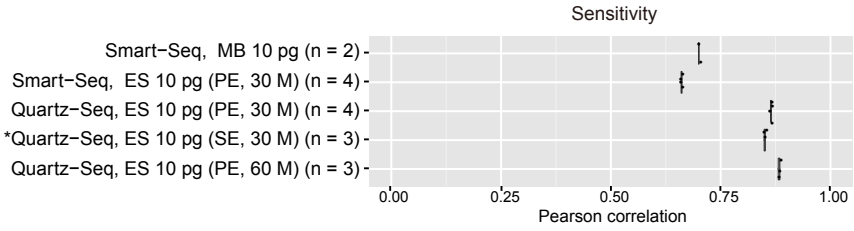

a
Reproducibility
y = 0.94x + 0.02
R = 0.935
7994 of 9811 (81.5 %)
ES 10 pg total RNA (WTA) #2
log10(FPKM+1)
ES 10 pg total RNA (WTA) #1
log10(FPKM+1)

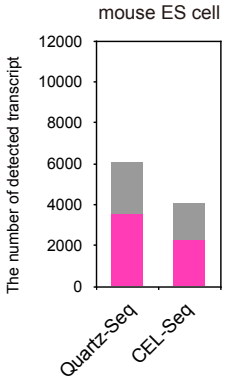

Sensitivity
y = 0.9x + 0.04
R = 0.895
6515 of 9634 (67.6 %)
ES 10 pg total RNA (WTA) #1
log10(FPKM+1)
ES 1 μg total RNA (Non-WTA)
log10(FPKM+1)

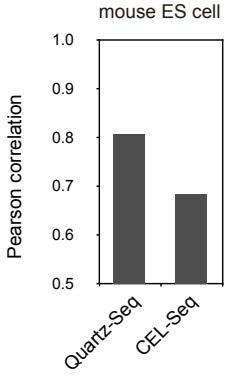

b
Reproducibility
The number of detected transcript
non-WTA
Quartz-Seq
Smart-Seq


Sensitivity
The number of detected transcript
Quartz-Seq
Smart-Seq


Reproducibility
Smart−Seq (Nx), UHRR 10 pg (n = 6)
Smart−Seq, UHRR 10 pg (n = 6)
Smart−Seq (Nx), HB 10 pg (n = 5)
Smart−Seq, MB 10 pg (n = 2)
Smart−Seq, ES 10 pg (PE, 30 M) (n = 4)
Quartz−Seq, ES 10 pg (PE, 30 M) (n = 4)
*Quartz−Seq, ES 10 pg (SE, 30 M) (n = 3)
Quartz−Seq, ES 10 pg (PE, 60 M) (n = 3)
Non−WTA (n = 2)
Pearson correlation


Sensitivity
Smart−Seq, MB 10 pg (n = 2)
Smart−Seq, ES 10 pg (PE, 30 M) (n = 4)
Quartz−Seq, ES 10 pg (PE, 30 M) (n = 4)
*Quartz−Seq, ES 10 pg (SE, 30 M) (n = 3)
Quartz−Seq, ES 10 pg (PE, 60 M) (n = 3)
Pearson correlation


c
mouse ES cell
The number of detected transcript
Quartz-Seq
CEL-Seq


mouse ES cell
Pearson correlation
Quartz-Seq
CEL-Seq