THE OHIO STATE UNIVERSITY

First decade of single-cell sequencing

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January 22, 2021

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Milestones of single cell sequencing since 2009

Transcriptome

2009: scRNA-seq


nature methods
Published: 06 April 2009

mRNA-Seq whole-transcriptome analysis of a single cell

Fuchou Tang, Catalin Barbacioru, Yangzhou Wang, Ellen Nordman, Clarence Lee, Nanlan Xu, Xiaohui Wang, John Bodeau, Brian B Tuch, Asim Siddiqui, Kaiqin Lao & M Azim Surani

Genome

2011: scWGS


nature
Published: 13 March 2011

Tumour evolution inferred by single-cell sequencing

Nicholas Navin, Jude Kendall, Jennifer Troge, Peter Andrews, Linda Rodgers, Jeanne McIndoo, Kerry Cook, Asya Stepansky, Dan Levy, Diane Esposito, Lakshmi Muthuswamy, Alex Krasnitz, W. Richard McCombie, James Hicks & Michael Wigler

2012: scExome-seq


Cell

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Single-Cell Exome Sequencing Reveals Single-Nucleotide Mutation Characteristics of a Kidney Tumor

Xun Xu · Yong Hou · Xuyang Yin · ... Michael Dean · Yingrui Li · Jun Wang

Multiomics

2015: scG&T (genome & transcriptome)


nature methods

G&T-seq: parallel sequencing of single-cell genomes and transcriptomes

Iain C Macaulay, Wilfried Haerty, Parveen Kumar, Yang I Li, Tim Xiaoming Hu, Mabel J Teng, Mubeen Goolam, Nathalie Saurat, Paul Coupland, Lesley M Shirley, Miriam Smith, Niels Van der Aa, Ruby Banerjee, Peter D Ellis, Michael A Quail, Harold P Swerdlow, Magdalena Zernicka-Goetz, Frederick J Livesey, Chris P Ponting & Thierry Voet

2018: scNMT-seq (transcriptome & epigenome)


nature communications
Article | Open Access | Published: 22 February 2018

scNMT-seq enables joint profiling of chromatin accessibility DNA methylation and transcription in single cells

Stephen J. Clark, Ricard Argelaguet, Chantriolnt-Andreas Kapourani, Thomas M. Stubbs, Heather J. Lee, Celia Alda-Catalinas, Felix Krueger, Guido Sanguinetti, Gavin Kelsey, John C. Marioni, Oliver Stegle & Wolf Reik

2017: scTrio-seq (genome & transcriptome & epigenome


Cell Research
Open Access | Published: 16 June 2017

Single-cell multi-omics sequencing of mouse early embryos and embryonic stem cells

Fan Guo, Lin Li, Jingyun Li, Xinglong Wu, Boqiang Hu, Ping Zhu, Lu Wen & Fuchou Tang

Epigenome

2013: scHi-C

nature
Published: 25 September 2013

Single-cell Hi-C reveals cell-to-cell variability in chromosome structure

Takashi Nagano, Yaniv Lubling, Tim J. Stevens, Stefan Schoenfelder, Eitan Yaffe, Wendy Dean, Ernest D. Laue, Amos Tanay & Peter Fraser

2015: scChIP-seq

nature biotechnology
Published: 12 October 2015

Single-cell ChIP-seq reveals cell subpopulations defined by chromatin state

Assaf Rotem, Oren Ram, Noam Shoresh, Ralph A Sperling, Alon Goren, David A Weitz & Bradley E Bernstein

2015: scATAC-seq


nature
Published: 17 June 2015

Single-cell chromatin accessibility reveals principles of regulatory variation

Jason D. Buenrostro, Beijing Wu, Ulrike M. Litzenburger, Dave Ruff, Michael L. Gonzales, Michael P. Snyder, Howard Y. Chang & William J. Greenleaf

2017: scNOMe-seq

Simultaneous measurement of chromatin accessibility, DNA methylation, and nucleosome phasing in single cells

Sebastian Pott*

2017: scCOOL-seq


Cell Research
Open Access | Published: 16 June 2017

Single-cell multi-omics sequencing of mouse early embryos and embryonic stem cells

Fan Guo, Lin Li, Jingyun Li, Xinglong Wu, Boqiang Hu, Ping Zhu, Lu Wen & Fuchou Tang

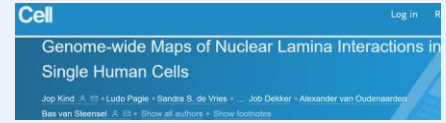
2015: scWGBS-seq


Cell Reports

Single-Cell DNA Methylome Sequencing and Bioinformatic Inference of Epigenomic Cell-State Dynamics

Matthias Farlik · Nathan C. Sheffield · Angelo Nuzzo · ... Andreas Schönegger · Johanna Klughammer · Christoph Bock · Show all authors · Show footnotes

2015: scDamID-seq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Genome-wide Maps of Nuclear Lamina Interactions in Single Human Cells

Jop Kind · Ludo Pagie · Sandra S. de Vries · ... Job Dekker · Alexander van Oudenaarden · Bas van Steensel · Show all authors · Show footnotes

2015: scDNase-seq


nature
Published: 25 November 2015

Genome-wide detection of DNase I hypersensitive sites in single cells and FFPE tissue samples

Wenfei Jin, Qingsong Tang, Mimi Wan, Kairong Cui, Yi Zhang, Gang Ren, Bing Ni, Jeffrey Sklar, Teresa M. Przytycka, Richard Childs, David Levens & Keji Zhao

2018: scMNase-seq


nature
Letter | Published: 26 September 2018

Principles of nucleosome organization revealed by single-cell micrococcal nuclease sequencing

Binbin Lai, Weiwu Gao, Kairong Cui, Wanli Xie, Qingsong Tang, Wenfei Jin, Gangqing Hu, Bing Ni & Keji Zhao

2019: scCUT&RUN

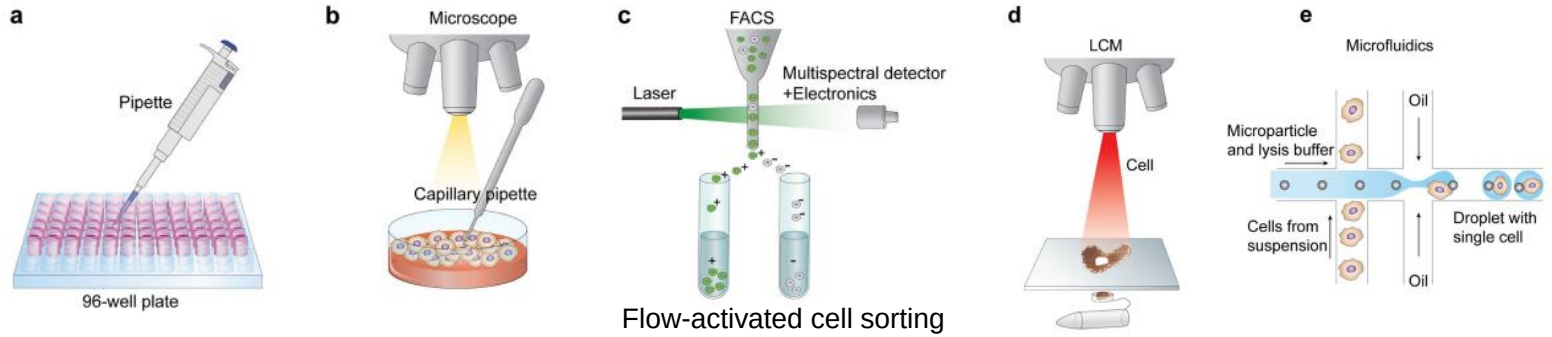

Cell

Profiling of Pluripotency Factors in Single Cells and Early Embryos

Sarah J. Hainer · Ana Boškovic · Kurtis N. McCannell · Oliver J. Rando · Thomas G. Fazzio

Typical single-cell experimental/technical pipeline

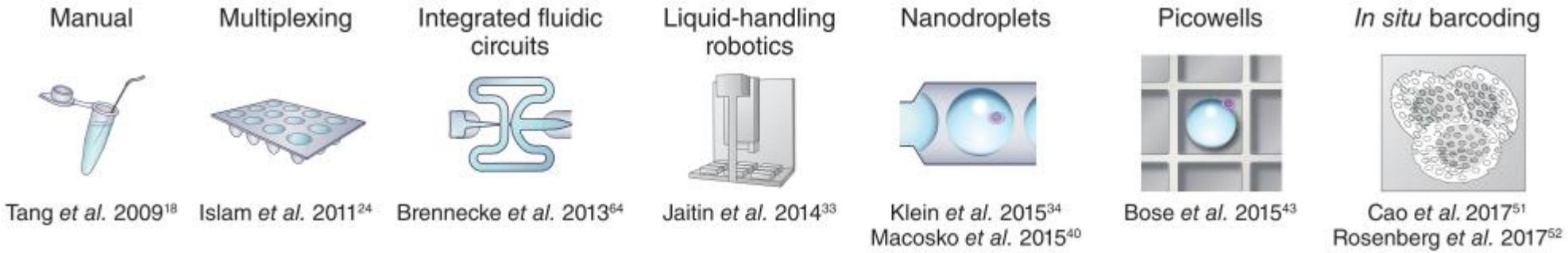
Step 1: Single cell isolation

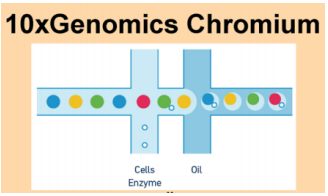


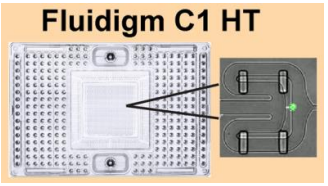
Flow-activated cell sorting

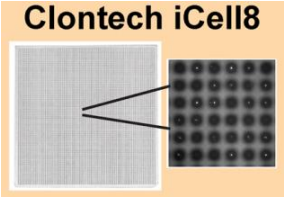
Laser capture microdissection

Step 2: Library preparation (cell capture and lysis, PCR-based barcoding)





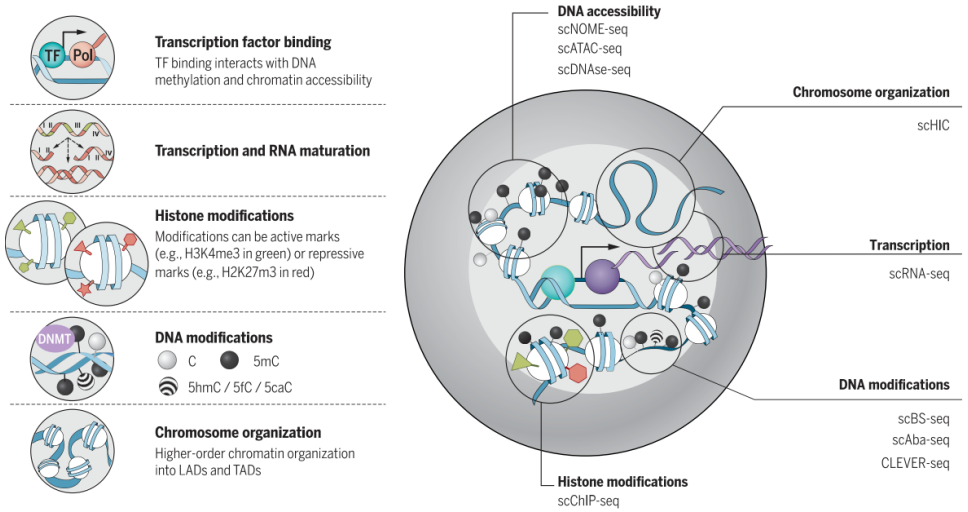




Step 3: Sequencing

Hwang, et al. *Exp Mol Med* (2018); Wang, et al. *bioRxiv* (2019); Svensson, et al. *Nat Protoc* (2018)

More challenges for single-cell genome/epigenome sequencing

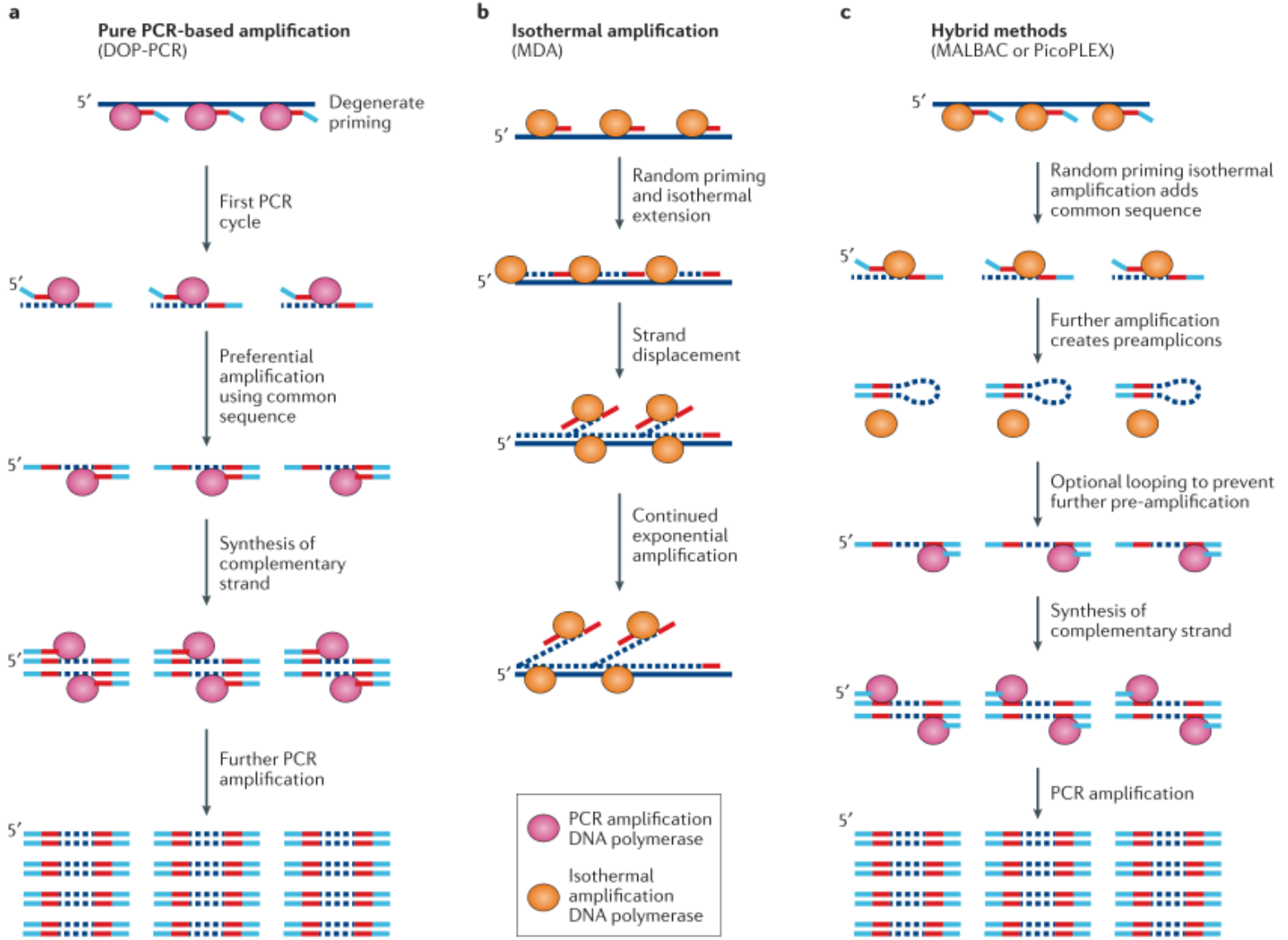


Development of scRNA-seq

- **A single diploid human cell (~3GB genome size)**
  - **DNA**
    - Two copies: paternal and maternal
    - ~6 pg
  - **RNA**
    - 360,000 mRNA molecules; 12,000 different transcripts
    - 10-30 pg total RNA, 1-5% of them are mRNA
- **Sequencer**
  - **Nanogram – microgram**
- **Solution**
  - **PCR amplification**
  - **Pool many single cells – cell barcoding**
    - **PCR-based barcoding**
    - **PCR-free barcoding (transposase)**

Kelsey, et al. *Science* (2017); Svensson, et al. *Nat Protoc* (2018)

Typical single-cell genome/epigenome sequencing: amplification first



	PCR-based (DOP-PCR)	Isothermal (MDA)	Hybrid (MALBAC or PicoPLEX)
False-negative rate (coverage and allelic dropout)	High	Low	Intermediate
Non-uniformity	Low	High	Low
False-positive rate (amplification error rate)	High	Low	Intermediate

Lähnemann *et al. Genome Biology* (2020) 21:31
https://doi.org/10.1186/s13059-020-1926-6

Genome Biology

REVIEW Open Access

Eleven grand challenges in single-cell data science

David Lähnemann[1,2,3], Johannes Köster[1,4], Ewa Szczurek[5], Davis J. McCarthy[6,7], Stephanie C. Hicks[8], Mark D. Robinson[9], Catalina A. Vallejos[10,11], Kieran R. Campbell[12,13,14], Niko Beerenwinkel[15,16], Ahmed Mahfouz[17,18], Luca Pinello[19,20,21], Pavel Skums[22], Alexandros Stamatakis[23,24], Camille Stephan-Otto Attolini[25], Samuel Aparicio[13,26], Jasmijn Baaijens[27], Marleen Balvert[27,28], Buys de Barbanson[29,30,31], Antonio Cappuccio[32], Giacomo Corleone[33], Bas E. Dutilh[28,34], Maria Florescu[29,30,31], Victor Guryev[35], Rens Holmer[36], Katharina Jahn[15,16], Thamar Jessurun Lobo[35], Emma M. Keizer[37], Indu Khatri[38], Szymon M. Kielbasa[39], Jan O. Korbel[40], Alexey M. Kozlov[23], Tzu-Hao Kuo[3], Boudewijn P.F. Lelieveldt[41,42], Ion I. Mandoiu[43], John C. Marioni[44,45,46], Tobias Marschall[47,48], Felix Mölder[1,49], Amir Niknejad[50,51], Lukasz Raczkowski[5], Marcel Reinders[17,18], Jeroen de Ridder[29,30], Antoine-Emmanuel Saliba[52], Antonios Somarakis[42], Oliver Stegle[40,46,53], Fabian J. Theis[54], Huan Yang[55], Alex Zelikovsky[56,57], Alice C. McHardy[3], Benjamin J. Raphael[58], Sohrab P. Shah[59] and Alexander Schönhuth[27,28*]

- **Errors/issues during WGA**
  - **Loss of coverage**
    - MDA: 84%
    - MALBAC: 72%
    - DOP-PCR: 39%
  - **Decreased coverage uniformity**
  - **Allelic imbalance/allelic dropout**
  - **Errors during PCR amplification**

Gawad, et al. *Nat Rev Genet* (2016)

Single-cell genome sequencing with PCR-free barcoding

nature methods

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nature › nature methods › articles › article

Published: 09 January 2017

Scalable whole-genome single-cell library preparation without preamplification

Hans Zahn, Adi Steif, Emma Laks, Peter Eirew, Michael VanInsberghe, Sohrab P Shah, Samuel Aparicio & Carl L Hansen

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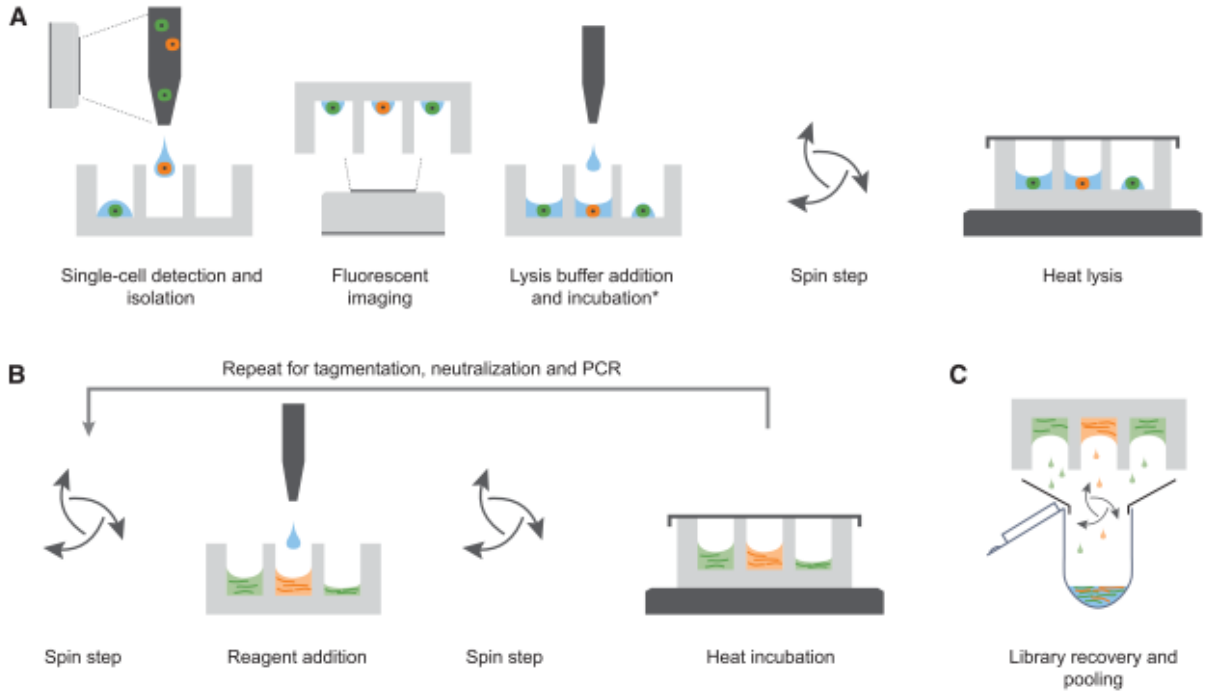
RESOURCE | VOLUME 179, ISSUE 5, P1207-1221.E22, NOVEMBER 14, 2019

PDF [10 MB]

Clonal Decomposition and DNA Replication States Defined by Scaled Single-Cell Genome Sequencing

Emma Laks [11] • Andrew McPherson [11] • Hans Zahn [11] • ... Carl Hansen • Sohrab P. Shah • Samuel Aparicio [12] • Show all authors • Show footnotes

Barcoding first, then PCR amplication

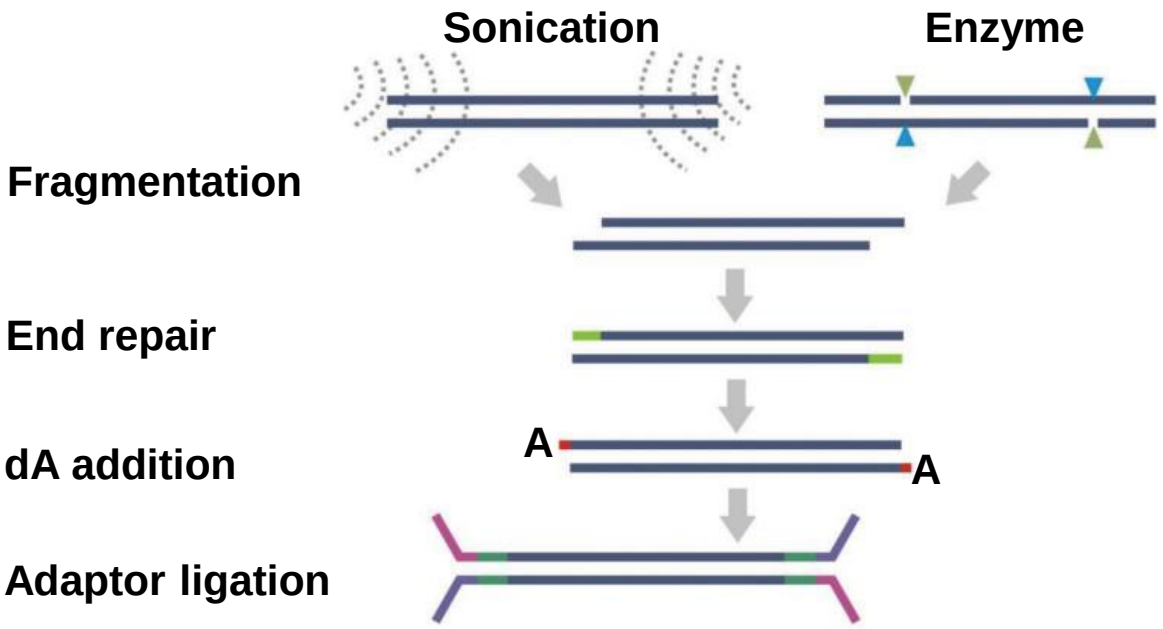


- Increase coverage uniformity compared to pre-amplification methods
  - Copy number variation analysis

- On average, <15% genome coverage per single cell

Two types of PCR-free barcoding methods

DNA ligase-based (more steps, low efficiency)



Transposase-based (simpler, faster, high efficiency)

Tn5 transposase complex with adaptors

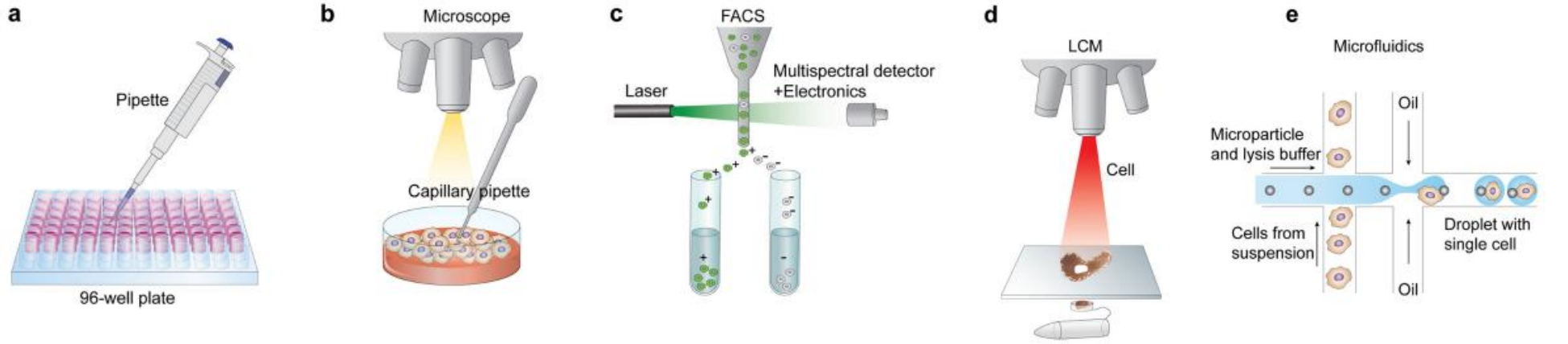
"Cut and Insert"

- **Single-cell single-molecule long-read sequencing (transposase-based barcoding + PacBio/ONT)**
  - **An automatic platform (e.g., 10X): large cell number**
  - **Cost: thousands of Tn5 complex with different barcodes**

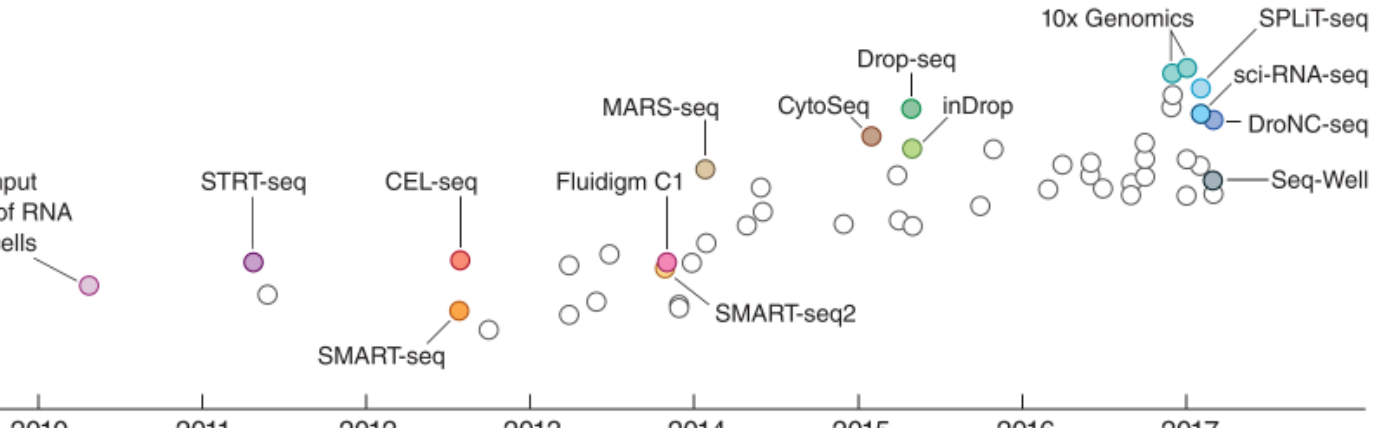
Adey, et al. *Genome Biol* (2010)

Thanks

Single-cell isolation



Manual	Multiplexing	Integrated fluidic circuits	Liquid-handling robotics	Nanodroplets	Picowells	*In situ* barcoding
Tang *et al.* 2009[18]	Islam *et al.* 2011[24]	Brennecke *et al.* 2013[64]	Jaitin *et al.* 2014[33]	Klein *et al.* 2015[34] Macosko *et al.* 2015[40]	Bose *et al.* 2015[43]	Cao *et al.* 2017[51] Rosenberg *et al.* 2017[52]



Hwang, et al. *Exp Mol Med* (2018); Svensson, et al. *Nat Protoc* (2018)