THE OHIO STATE UNIVERSITY

Single-cell sequencing technology

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Timeline of single cell sequencing milestones since 2009

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

2013: single-cell full-length RNA-seq

nature methods

Published: 22 September 2013

Smart-seq2 for sensitive full-length transcriptome profiling in single cells

Simone Picelli, Åsa K Björklund, Omid R Faridani, Sven Sagasser, Gösta Winberg & Rickard Sandberg

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

Log in

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

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

RESOURCE | VOLUME 10, ISSUE 8, P1386-1397, MARCH 03, 2015

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

Cell

Log in

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

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

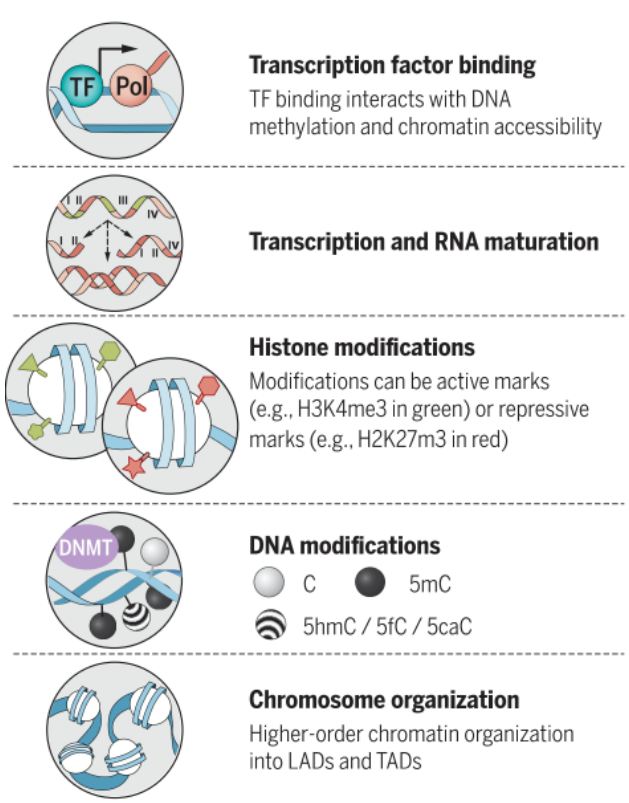
Cell Research

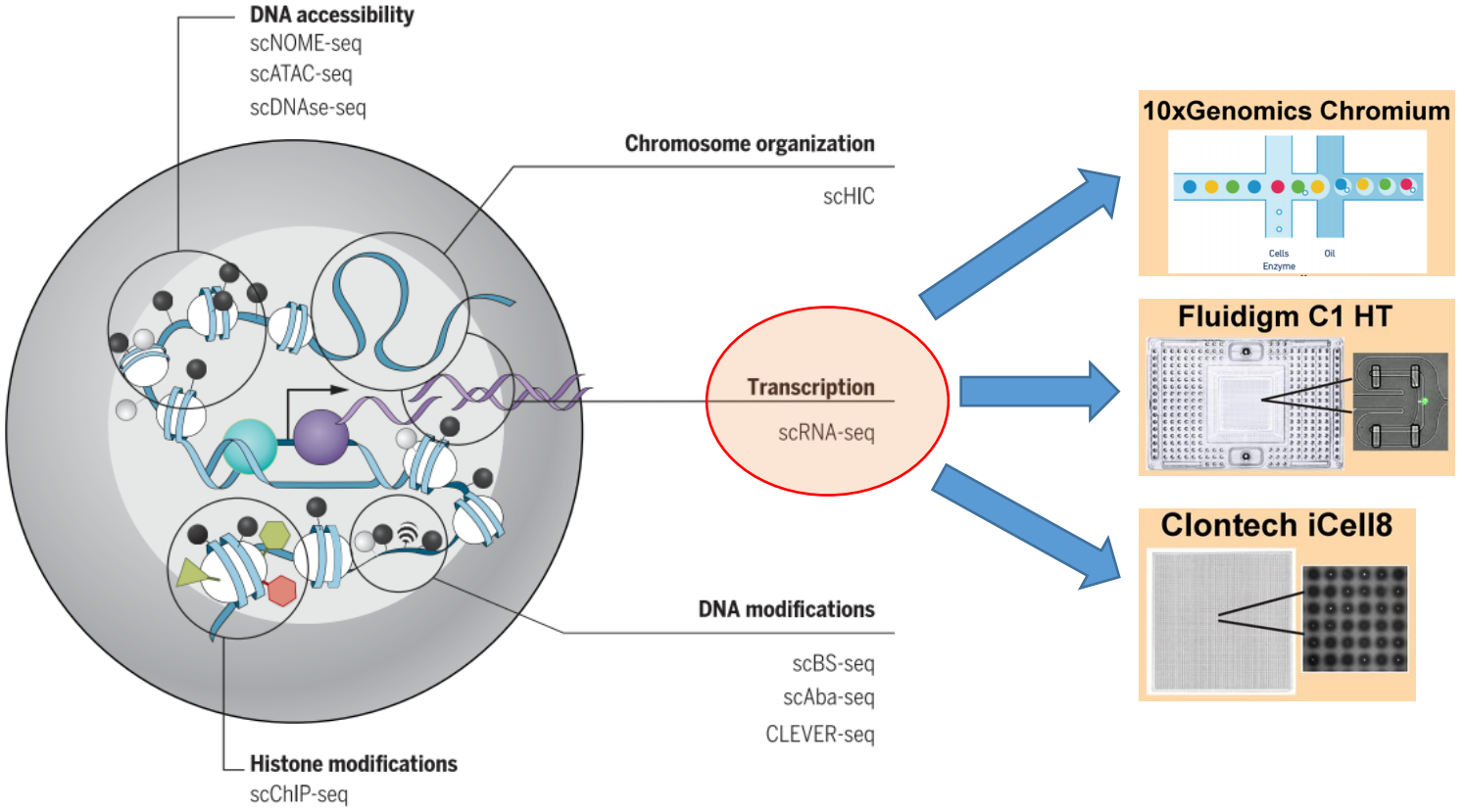
Open Access | Published: 16 June 2017

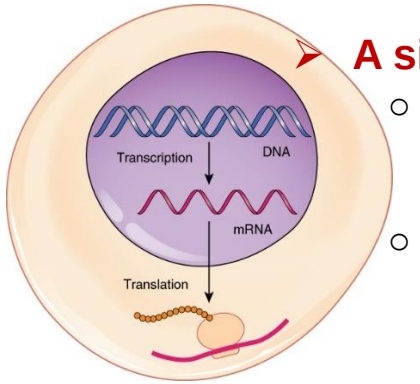
Single-cell multi-omics sequencing of mouse early embryos and embryonic stem cells

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

scRNA-seq *vs* single-cell genome/epigenome sequencing







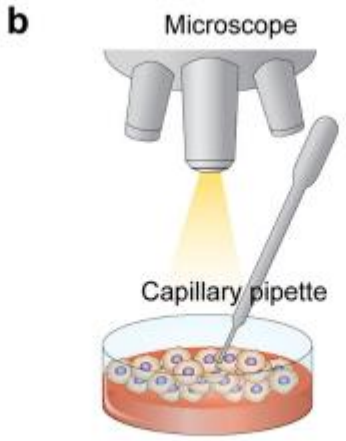
- **A single diploid mammalian cell**
  - **DNA**
    - Two copies: paternal and maternal
    - ~6 pg
  - **RNA**
    - 360,000 mRNA molecules; 12,000 different transcripts
    - 10-30 pg total RNA, 1-5% of them are mRNA

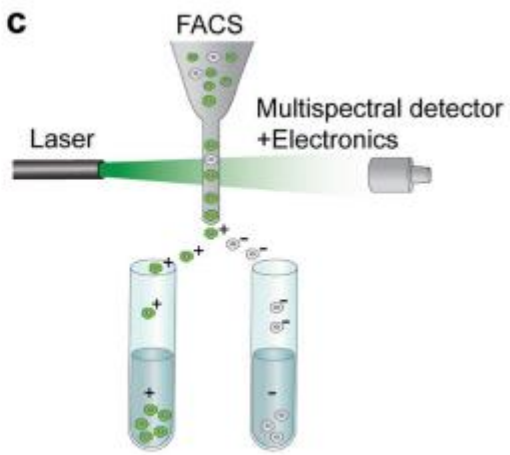
- **Sequencing usually requires**
  - **Nanogram – microgram**

- **How to perform single-cell sequencing?**
  - **PCR amplification**
  - **Pull single cells together (barcoding)**

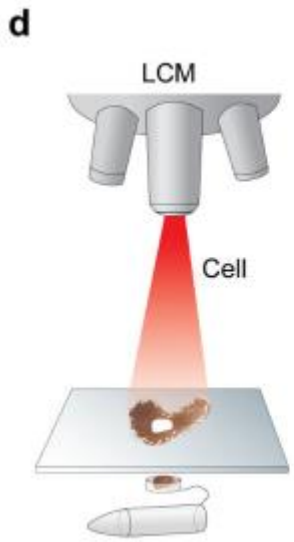
Kelsey, et al. *Science* (2017); Wang, et al. *bioRxiv* (2019); https://open.oregonstate.education/aandp/chapter/3-4-protein-synthesis/

Single-cell isolation

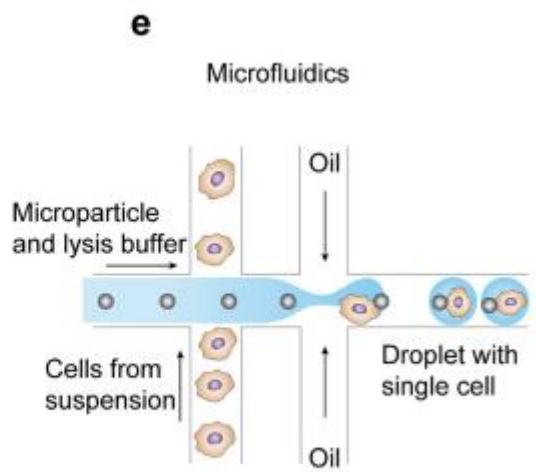




Flow-activated cell sorting

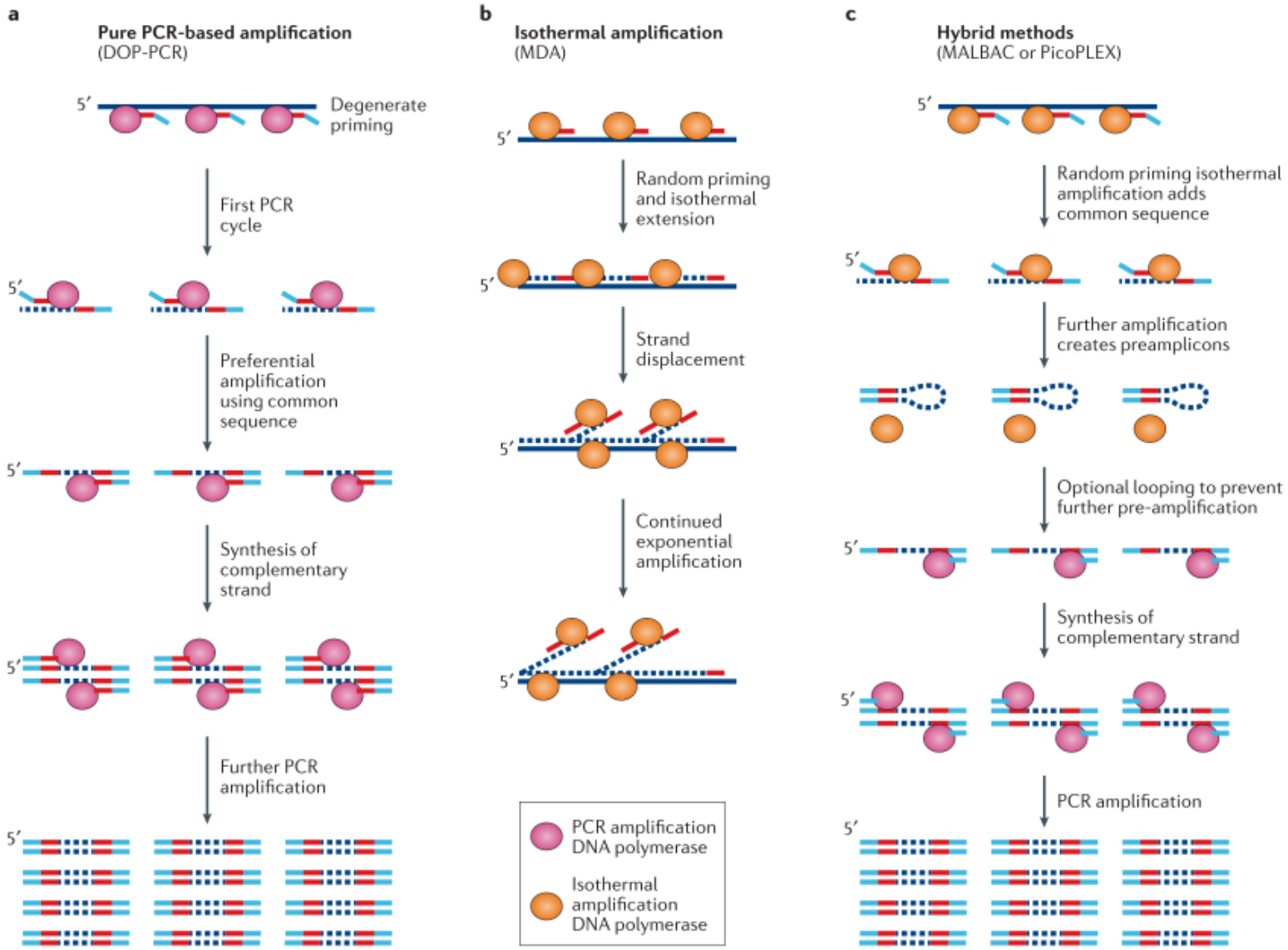


Laser capture microdissection



Hwang, et al. *Exp Mol Med* (2018)

Whole-genome amplification + Cell barcoding



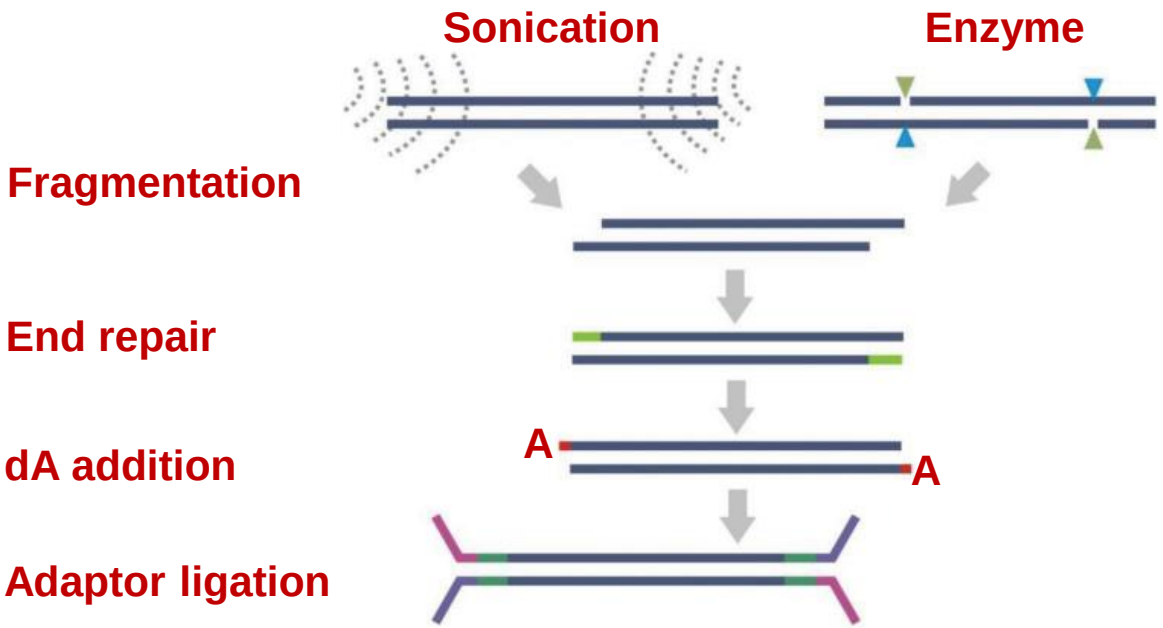
	PCR-based (DOP-PCR)	Isothermal (MDA)	Hybrid (MALBAC or PicoPLEX)
False-negative rate (coverage and allelic dropout)	High	Low	Intermediate
Non-uniformity	Low	High	Low
False-positive rate (amplification error rate)	High	Low	Intermediate

- **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)

Barcoding in a PCR-free way

DNA ligase-based



Transposase-based (simper, faster)

Tn5 transposase complex with adaptors

“Cut and Insert”

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

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

Thanks