

Introduction to DNA methylation and WGBS

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Outline

- Introduction to epigenome and DNA methylation
- Introduction to bisulfite sequencing
- Introduction to whole genome bisulfite sequencing (WGBS) data analysis

Epigenome

Genome Biology

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Research | [Open Access](#) | [Published: 15 September 2020](#)

Cancer-specific CTCF binding facilitates oncogenic transcriptional dysregulation

[Celestia Fang](#), [Zhenjia Wang](#), [Cuijuan Han](#), [Stephanie L. Safgren](#), [Kathryn A. Helmin](#), [Emmalee R. Adelman](#), [Valentina Serafin](#), [Giuseppe Basso](#), [Kyle P. Eagen](#), [Alexandre Gaspar-Maia](#), [Maria E. Figueroa](#), [Benjamin D. Singer](#), [Aakrosh Ratan](#), [Panagiotis Ntziachristos](#) & [Chongzhi Zang](#)

[Genome Biology](#) 21, Article number: 247 (2020) | [Cite this article](#)

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Early Detection of Ovarian Cancer Through Epigenetic Factors in the WHI

Ovarian cancer is the 5th leading cause of cancer death among US women; 62% of cases are diagnosed at advanced stages in which only 27% survive past 5 years. Yet, when ovarian cancer is detected at the localized stage (15% of cases), the 5 year survival rate is 94%. As survival is drastically improved if diagnosed early, methods to improve early detection through enhanced risk assessment, leading to better targeted primary prevention and screening are critical. The two validated risk assessment models from Rosner and Pfeiffer identify women at higher risk for ovarian cancer, but only have modest discriminatory power. Currently, no effective screening modality exists for ovarian cancer. Previous approaches for screening using single markers such as CA-125 in average risk populations have been unsuccessful, and led to many false positive results. Identifying novel panels of markers important to early carcinogenesis is key; epigenetic events (DNA methylation) are noted to occur early in carcinogenesis and reflect environmental insults and genetic vulnerability. Measurement of DNA methylation in cell free DNA has shown promise

Principal Investigator

Jeanine M. Genkinger

Awardee Organization

Columbia University Health Sciences

Fiscal Year

2020

Project End Date

02/28/2021

Funding Opportunity Announcement

PA-13-302

AGING


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Home > Archive > Volume 8, Issue 9 > DNA Methylation-based Measures Of Biolo...

Priority Research Paper

Volume 8, Issue 9

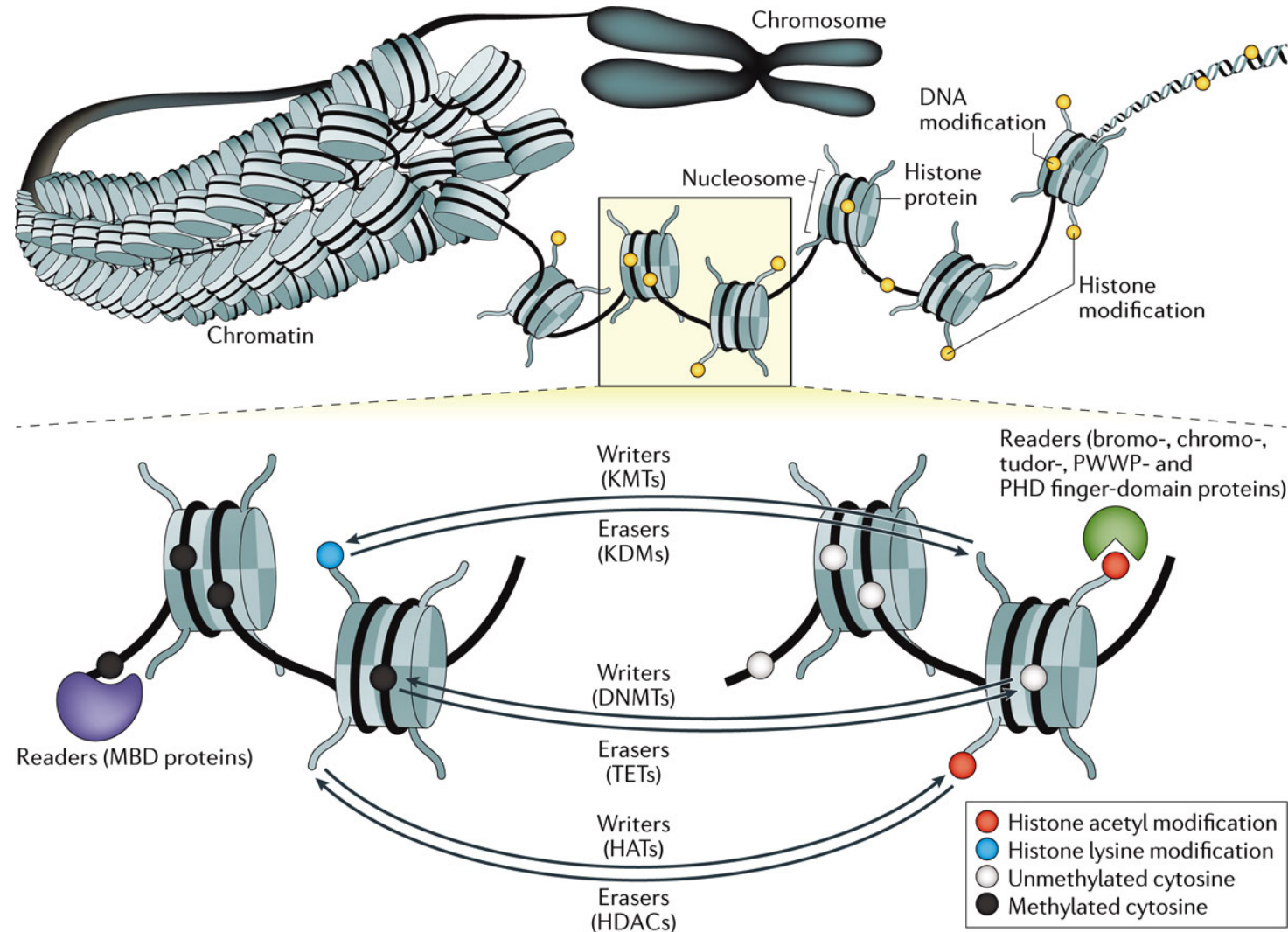
pp 1844—1865

970

DNA methylation-based measures of biological age: meta-analysis predicting time to death

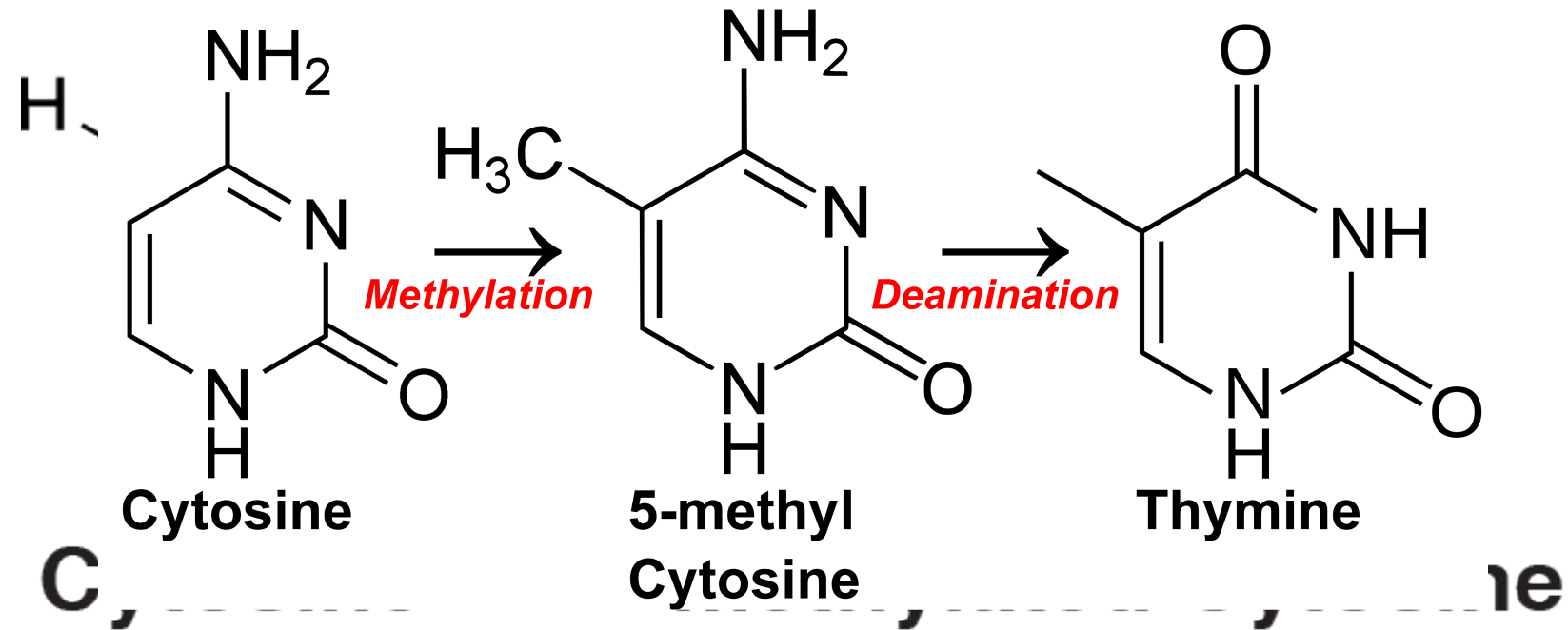
[* , Riccardo E. Marioni^{4,5,6, *}, Elena Colicino^{7, *}, ⁸, Cavin K. Ward-Caviness⁹, Pei-Chien Tsai¹⁰, ¹¹, Allan C. Just⁷, Ellen W. Demerath¹¹, Weihua Guan¹², ^{13,14}, Stephanie Studenski¹, Amy R. Vandiver¹⁵, ¹, Toshiko Tanaka¹, Douglas P. Kiel^{16,17}, Liming Liang^{18,19}, ¹⁸, Kathryn L. Lunetta^{2,20}, Joanne M. Murabito^{2,21},](#)

Epigenome

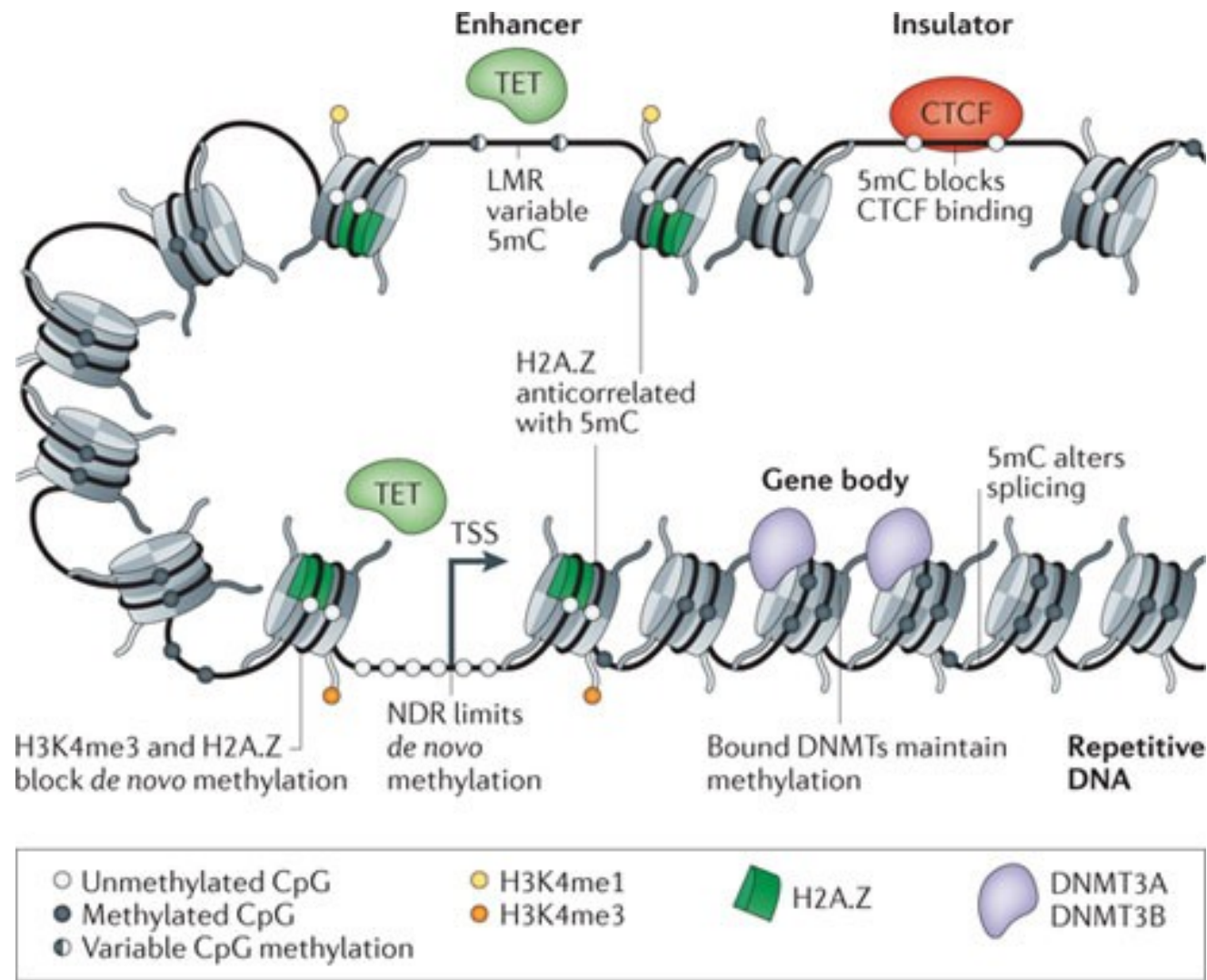


Nature Reviews | Genetics

DNA methylation

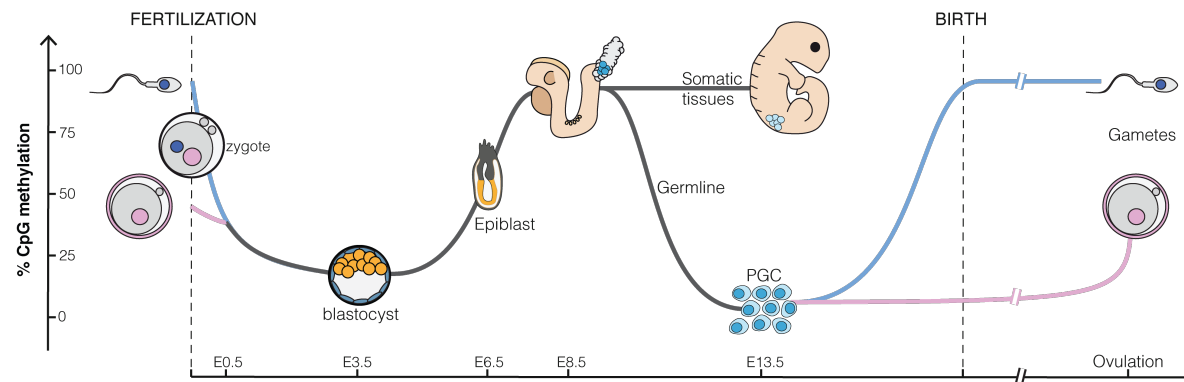


DNA methylation



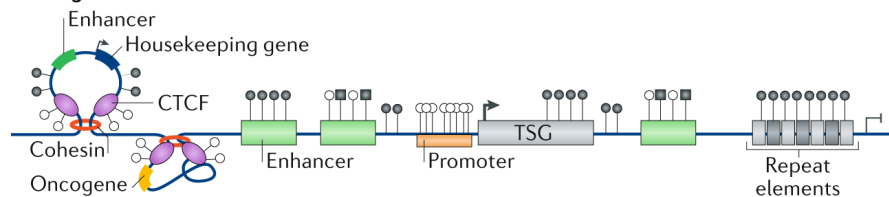
DNA methylation

- Regulates gene expression
 - Cellular development
 - Diseases

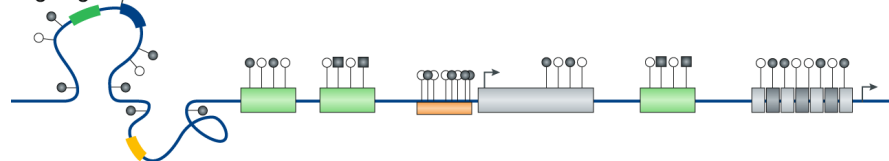


a DNA methylation

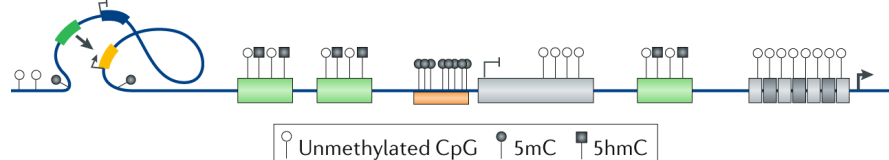
Young



Ageing



Cancer



b Histone methylation

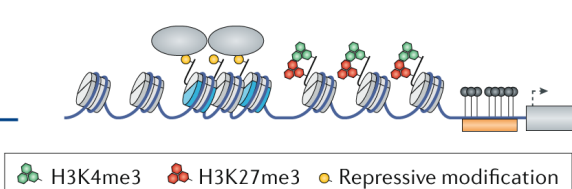
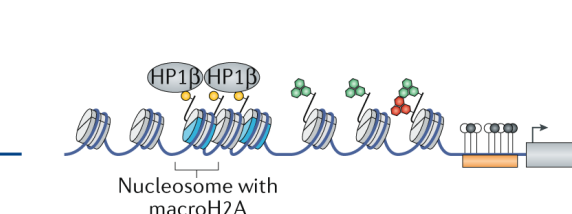
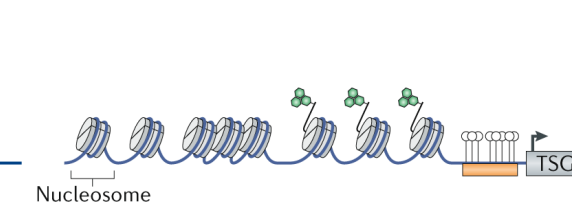
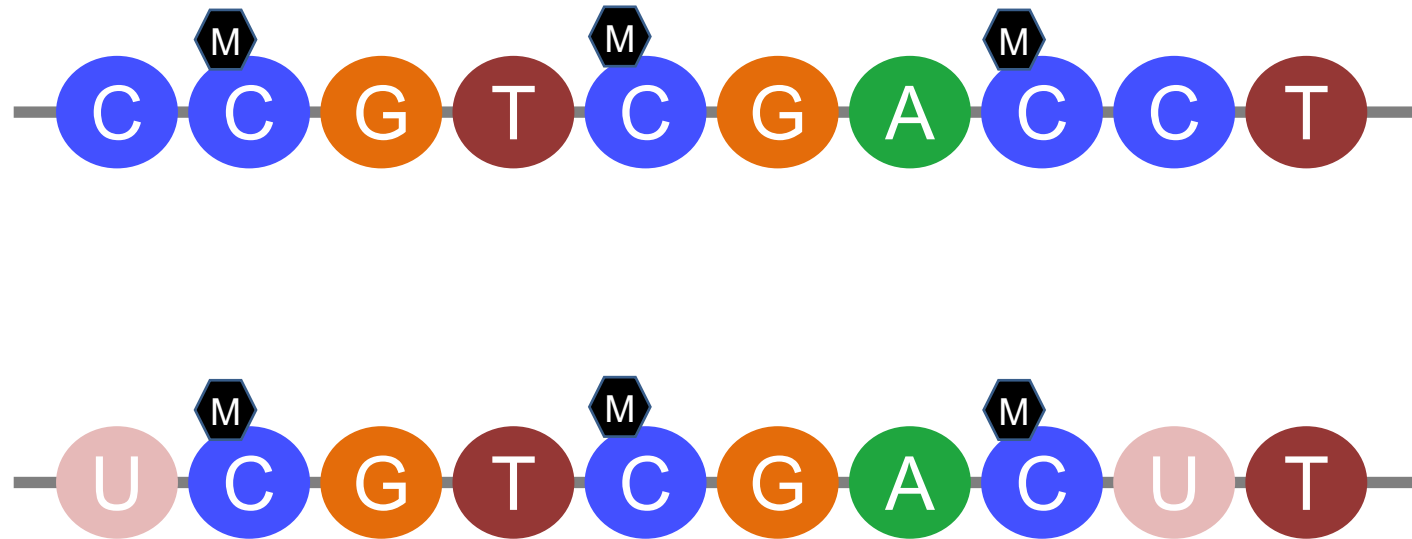


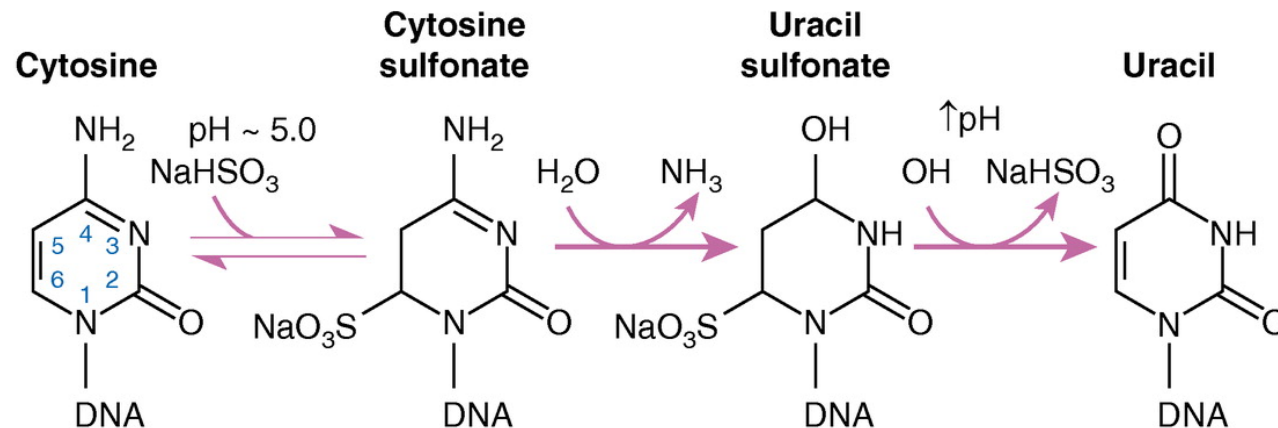
Image credit:

1. https://en.wikipedia.org/wiki/DNA_methylation
2. Michalak, E.M., Burr, M.L., Bannister, A.J. *et al.* The roles of DNA, RNA and histone methylation in ageing and cancer. *Nat Rev Mol Cell Biol* **20**, 573–589 (2019). <https://doi.org/10.1038/s41580-019-0143-1>

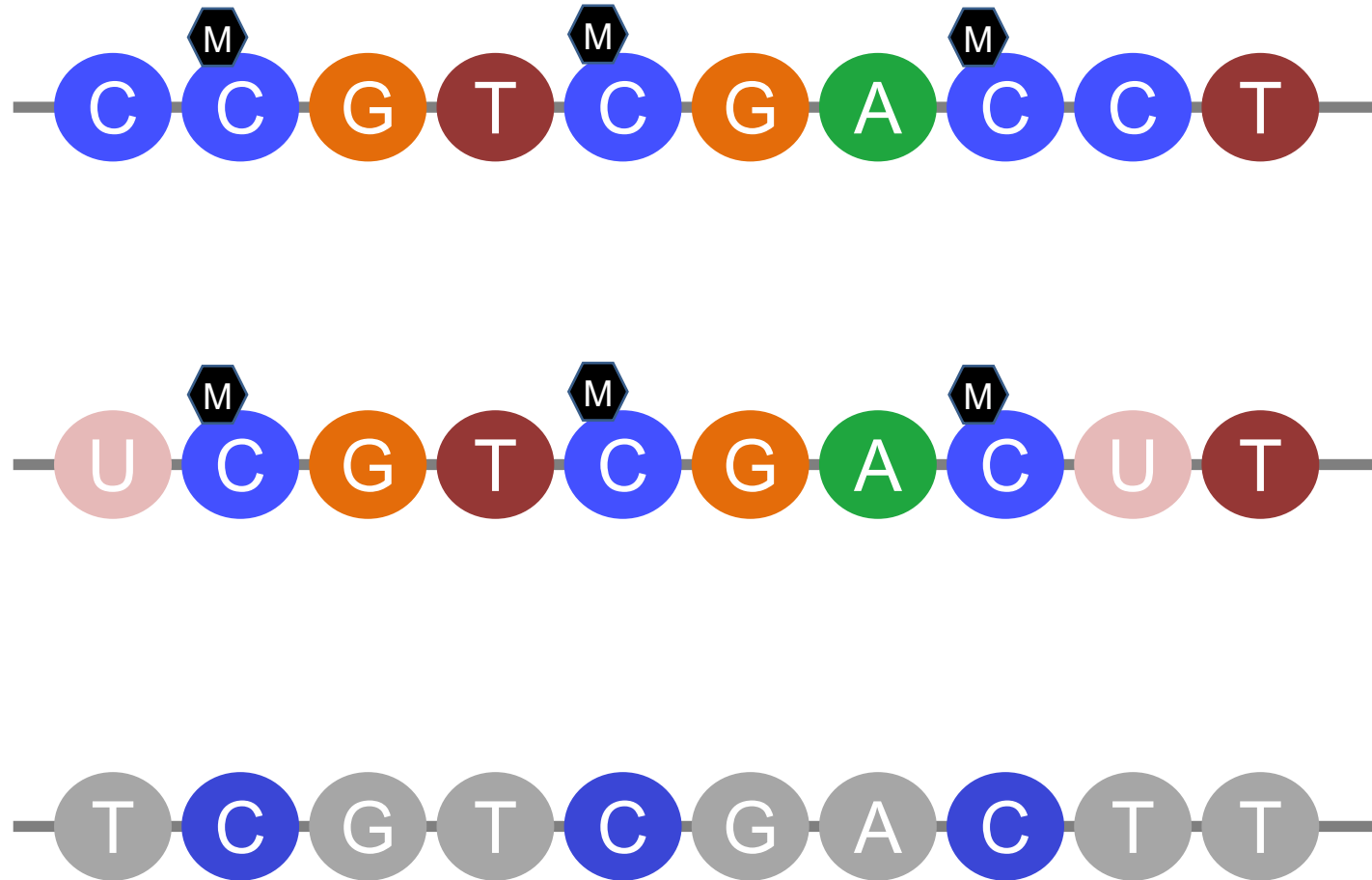
Bisulfite sequencing



**Bisulfite
conversion**



Bisulfite sequencing



**Bisulfite
conversion**

**PCR
Amplification
& Sequencing**

Compare to reference genome

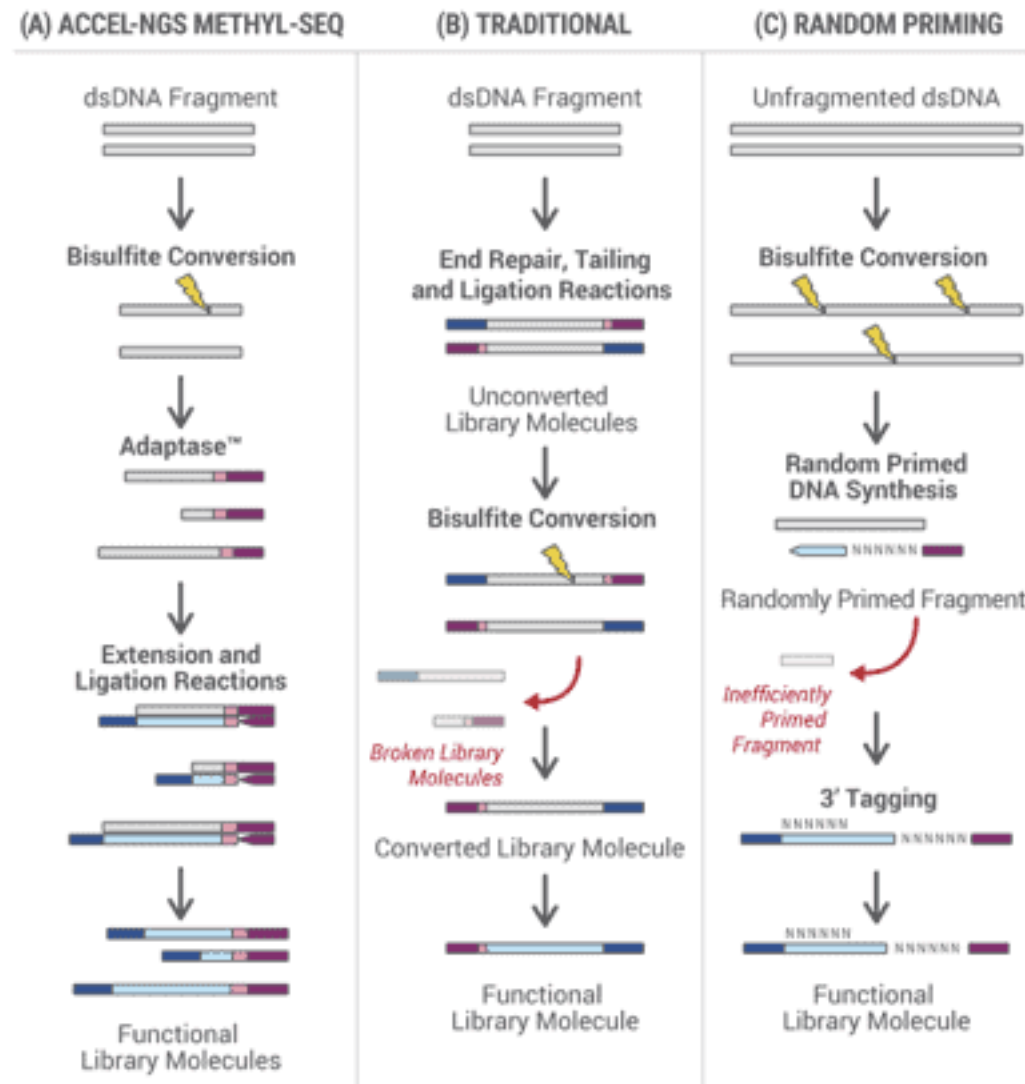
Bisulfite sequencing

- Whole genome bisulfite sequencing (WGBS)
 - Methylation levels genome wide
- Reduced representation bisulfite sequencing (RRBS)
 - Use restriction enzymes (MspI, to enrich for CpG rich regions of the genome (~1%))

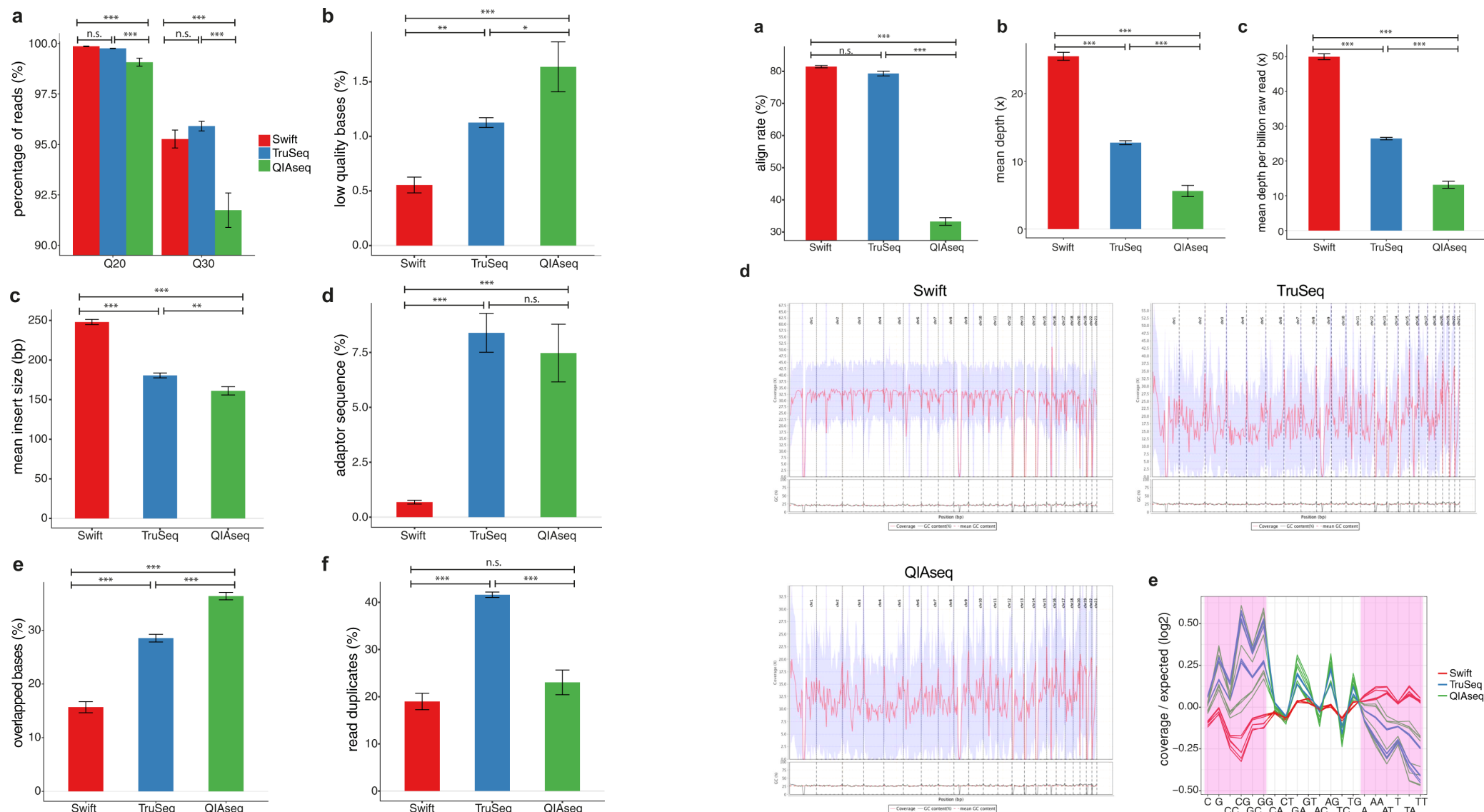
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WGBS library prep: Swift Biosciences



WGBS library prep: Swift Biosciences

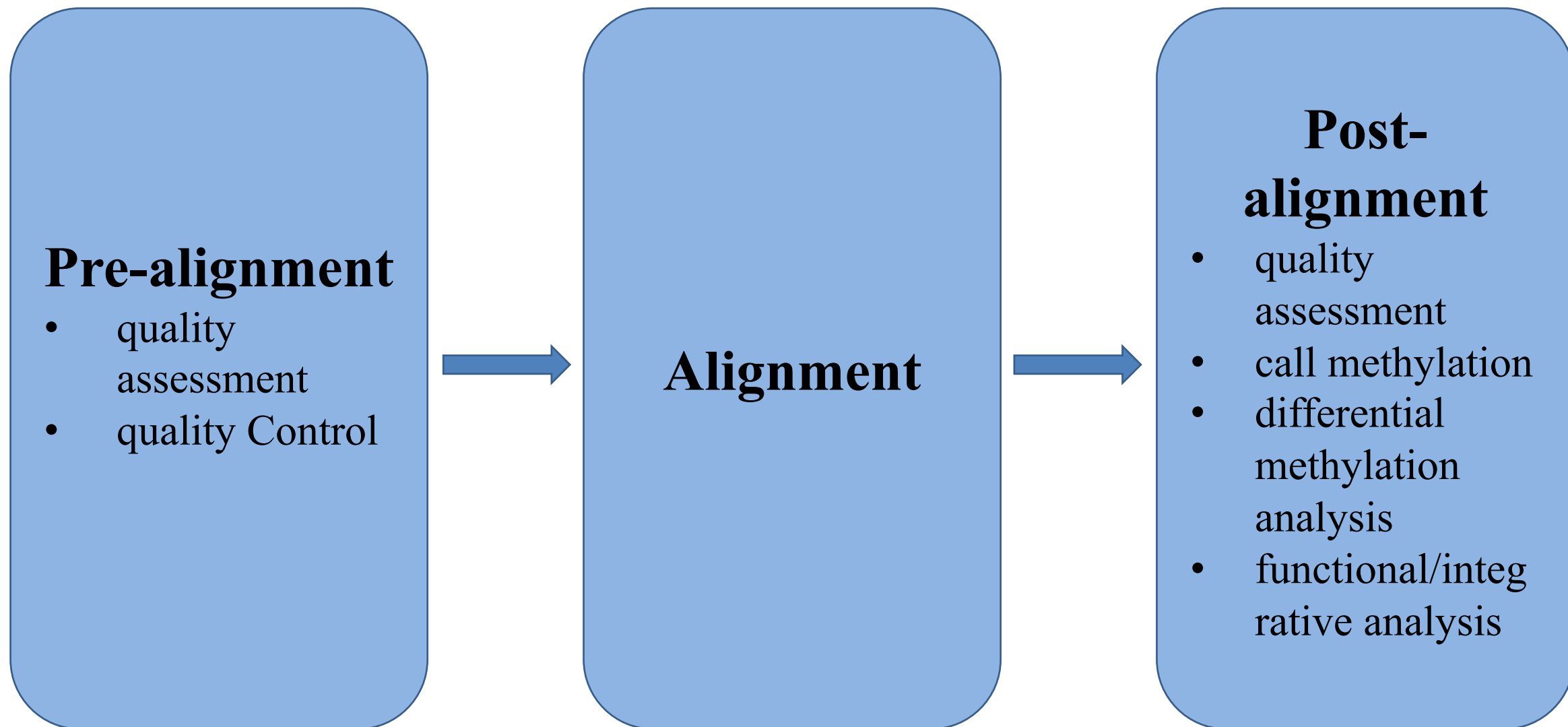


Zhou, L., Ng, H.K., Drautz-Moses, D.I. *et al.* Systematic evaluation of library preparation methods and sequencing platforms for high-throughput whole genome bisulfite sequencing. *Sci Rep* **9**, 10383 (2019). <https://doi.org/10.1038/s41598-019-46875-5>

WGBS study design

- Sequencing depth
 - In general, it depends on the magnitude of differences that one would like to detect: the larger the difference, the lower the coverage depth necessary.
 - ENCODE recommendation: 30X/replicate
 - Lower coverage can be used with the help of bioinformatics procedures
- Biological replicate
 - ENCODE recommends 2 biological replicates at minimum
 - DSS R package has developed an approach to detect differential methylation without biological replicates

WGBS data analysis workflow



WGBS data analysis workflow

Pre-alignment

- fastqc, multiqc
- trimgalore, trimmomatic
- ...



Alignment

- Bismark
- BS-Seeker2
- BSMAP
- bwa-meth
- ...

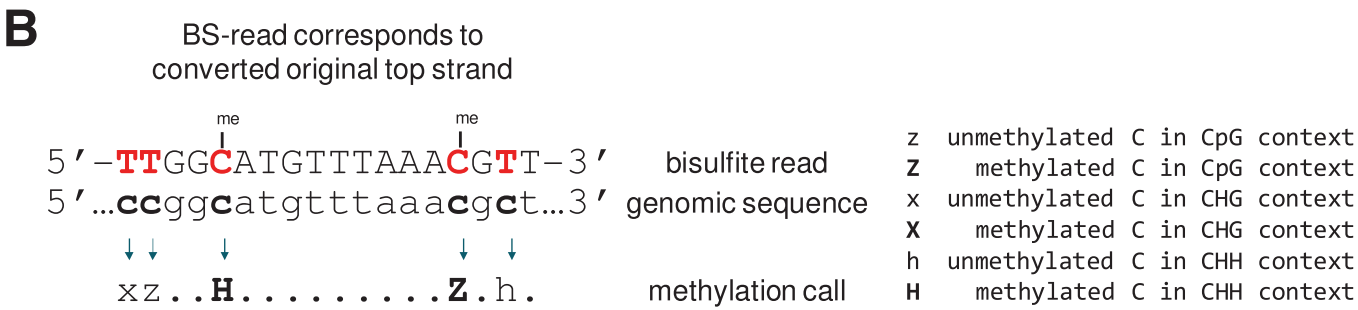
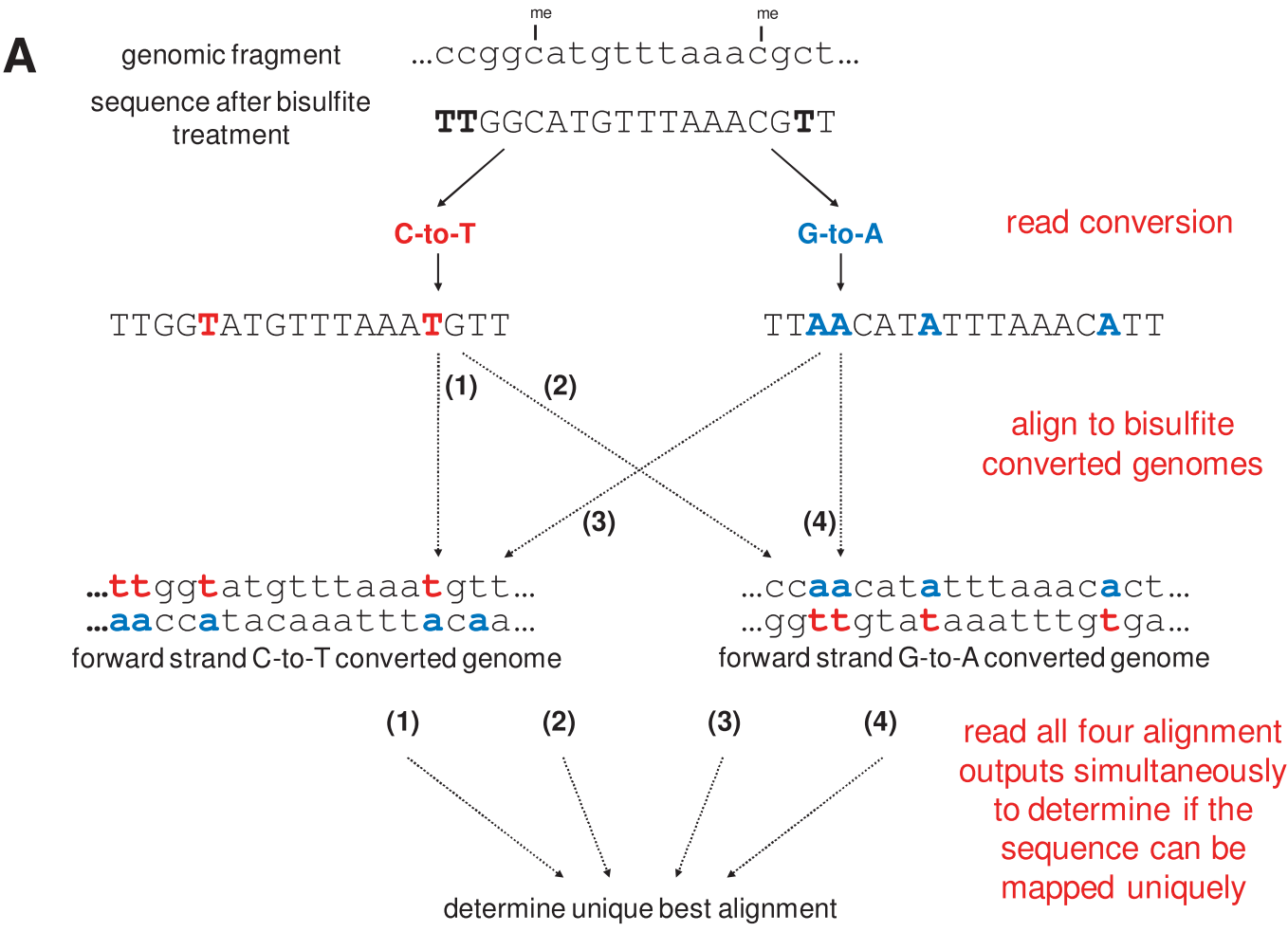


Post-alignment

- QualiMap
- Bismark
- DSS
- HOMER, deepTools
- ...

Bismark

Krueger F, Andrews SR. Bismark: a flexible aligner and methylation caller for Bisulfite-Seq applications. *Bioinformatics*. 2011;27(11):1571-1572. doi:10.1093/bioinformatics/btr167



Data used in the hands-on session


Cell Reports

Volume 31, Issue 3, 21 April 2020, 107525



Resource

Genome-wide Profiling Identifies DNA Methylation Signatures of Aging in Rod Photoreceptors Associated with Alterations in Energy Metabolism

Ximena Corso-Díaz¹, James Gentry¹, Ryan Rebernick¹, Catherine Jaeger¹, Matthew J. Brooks¹, Freekje van Asten¹, Keshav Kooragayala¹, Linn Gieser¹, Jacob Nellissery¹, Raul Covian², Tiziana Cogliati^{1, 3}, Anupam K. Mondal¹, Ke Jiang¹, Anand Swaroop^{1, 4}  

Questions ?