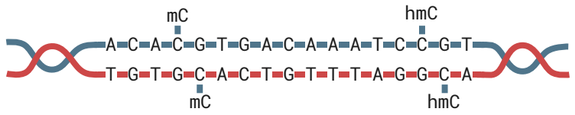
Joint genetic and epigenetic sequencing technology leads to improved genetics compared to existing methylation calling methods

Casper K Lumby[1,2], James Emery[2], Michael Gatzen[2], Christopher Kachulis[2], Megan Shand[2], Nicholas Harding[1], Jamie Scotcher[1], Shirong Yu[1], Páidí Creed[1], Eric Banks[2], Joanna D Holbrook[1]

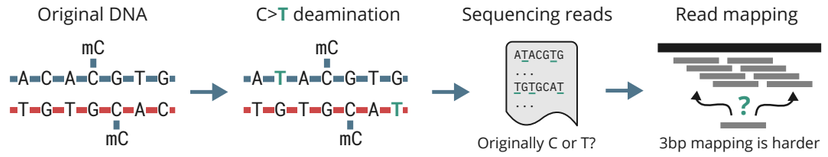
[1]Cambridge Epigenetix, Cambridge, United Kingdom | [2]Broad Institute of MIT and Harvard, Cambridge, MA, USA

Introduction

There is more to DNA than the genetic alphabet A, C, G and T. Epigenetics plays a causal role in cell fate, ageing and disease development. Methylated cytosines, such as 5mC and 5hmC, represent important biomarkers and are informally considered the 5th and 6th bases of DNA:



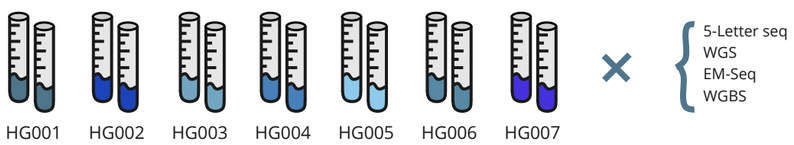
The combination of genetics and methylation has proved to be more powerful than either modality on their own. However, current methylation detection technologies rely on sacrificing genetic information for epigenetic insight:



We present a novel sequencing technology, **5-Letter seq**, that jointly determines genetics and methylation at high accuracy. In this poster we examine the **genetic** accuracy of the technology and benchmark it against existing methylation detection methods. This work derives from a collaboration between Cambridge Epigenetix and the Broad Institute.

Methodology

The Genome in a Bottle (GiaB) Consortium provides a complete genetic characterisation of 7 human samples (HG001-HG007). We sequenced all **seven** samples in **two replicates** across four technologies: Whole-genome sequencing (WGS), whole-genome bisulfite sequencing (WGBS), Enzymatic Methyl-seq (EM-Seq) and 5-Letter seq.



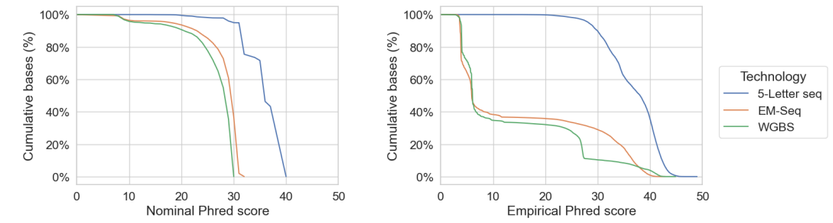
Phred Scores

Phred scores describe the accuracy of a base call, e.g. Q30 means that a base is 99.9% certain to be correctly called. We make two distinctions:

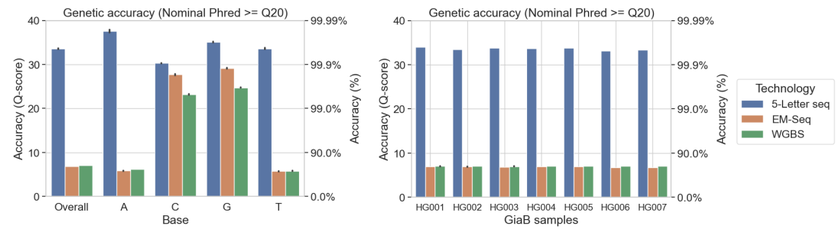
- *Nominal* Phred scores: These are accuracy **estimates** provided by the sequencing instrument. These may not be 100% accurate.
- *Empirical* Phred scores: These are accuracy **evaluations** obtained by comparing called bases with known bases.

Genetic Accuracy

Below are nominal and empirical Phred distributions. About 90% of 5-Letter seq bases have a Phred score greater than Q30 and around 35% have a score larger than Q40:



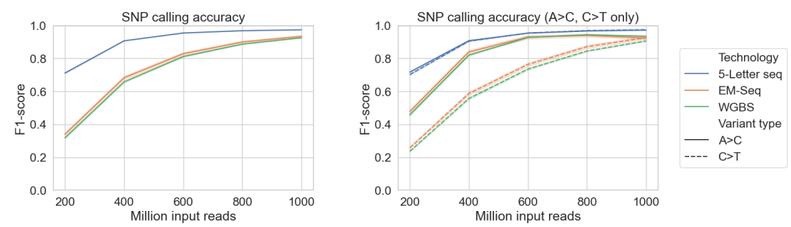
We can further stratify genetic accuracy by base type and GiaB sample:



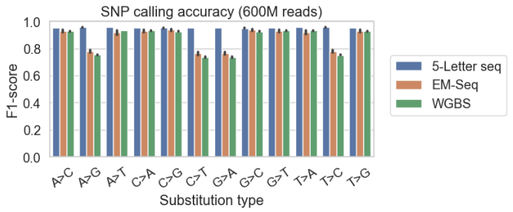
The accuracy of EM-Seq and WGBS is lower than that of 5-Letter seq. This is driven by C>T deamination, which results in reduced accuracy for T (forward strand) and A (reverse strand) bases, and read mapping using only 3 bases. Genetic accuracy is consistent across all 7 GiaB samples.

Variant Calling

SNP calling was performed using GATK4 for 5-Letter seq and using Bis-SNP for EM-Seq and WGBS. Evaluation showed that 5-Letter seq was significantly more accurate at variant calling than EM-Seq and WGBS:



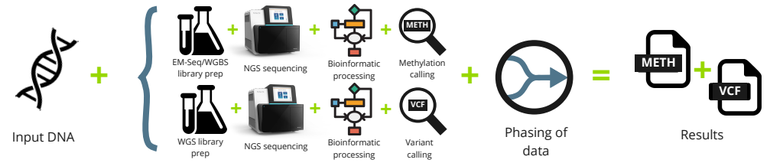
Additionally, 5-Letter seq performance was independent of SNP variant type:



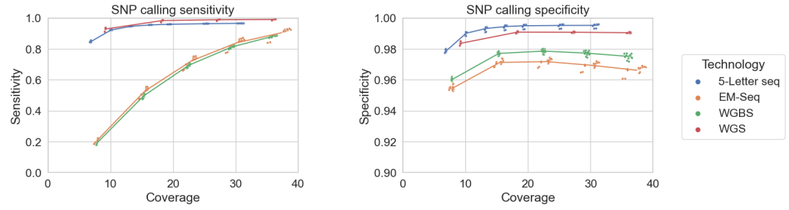
Impacted by C>T deamination	Variant type
Yes	C>T, T>C, A>G, G>A
No	A>C, A>T, C>A, C>G, G>C, G>T, T>A, T>G

Genetics + Epigenetics

With currently available methods, to achieve simultaneously high genetic and epigenetic accuracy, it is necessary to perform two separate workflows. This approach is limited by sample availability and the need for phasing data:



Under this setup, WGS variant calling is based on **half** the total sequencing volume. With 5-Letter seq, the above can be achieved with a single workflow. However, 5-Letter seq accomplishes this by resolving **two** reads into **one**. Therefore, SNP calling is compared here on coverage rather than number of input reads:



5L seq is more specific (0.5% more at 20X) and less sensitive (2.6% less at 20X) than WGS. Overall, 5-Letter seq produces highly accurate genetic and epigenetic calls. The phased nature of the data allows for generating novel insights (see other posters).

Additional 5-Letter Seq Posters

Cambridge Epigenetix and collaborators have additional posters at AGBT:

- **Poster 530**: *'Accurate and simultaneous sequencing of genetics and epigenetics in DNA'*, Creed et al.
- **Poster 414**: *'Profiling genetic and epigenetic changes, at read-level, after cellular rejuvenation'*, Holbrook et al.

Technology Paper and Communications

Technology paper: Füllgrabe, J. et al. (2023) 'Accurate simultaneous sequencing of genetic and epigenetic bases in DNA', *Nature Biotechnology.* Available at: https://www.nature.com/articles/s41587-022-01652-0

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