2017 LONDON CALLING

Measuring DNA Methylation with the MinION

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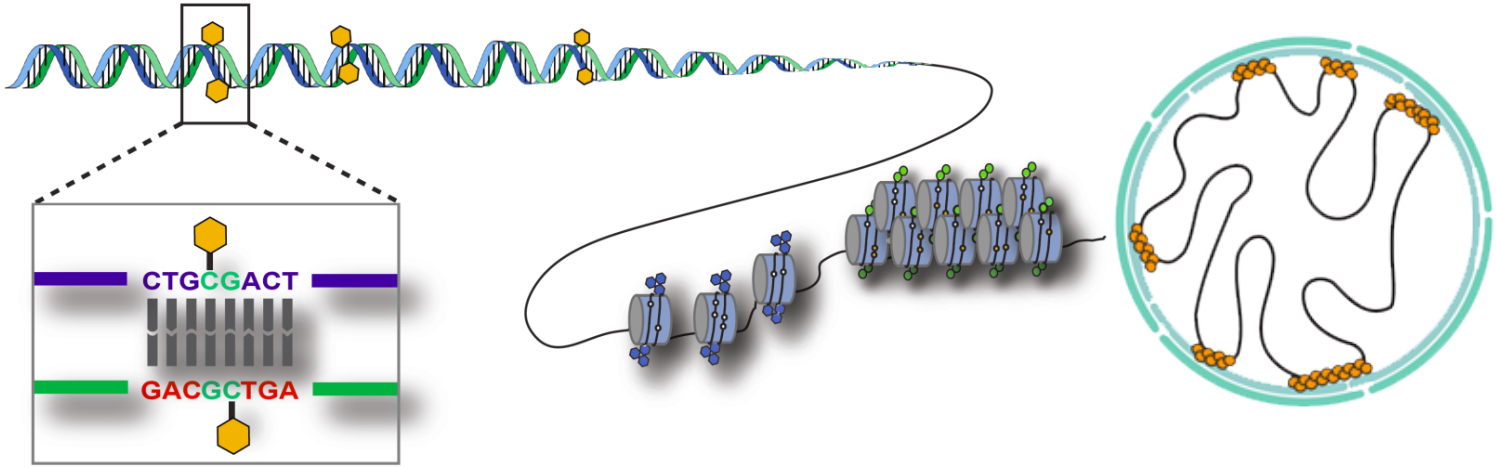
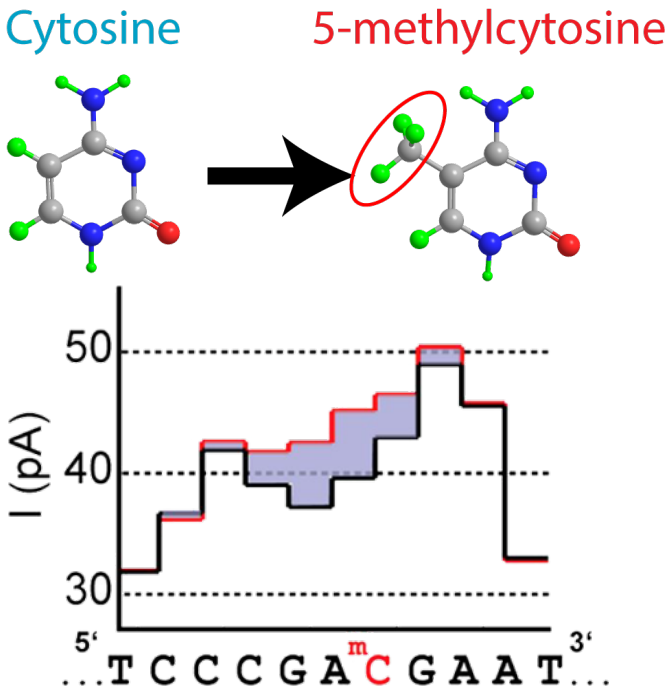
Epigenetics: Modern



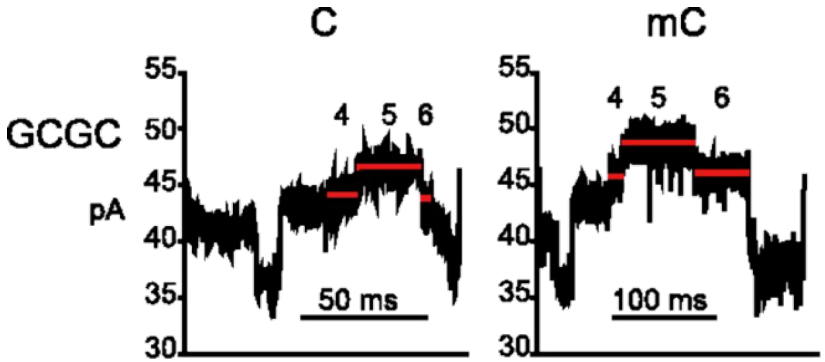
- Modern Definition of epigenetics involves heritable changes other than genetic sequence, e.g., positive feedback, high order structure, chromatin organization, histone modifications, DNA methylation.
- An analogy to a computer system:
  - DNA Sequence = Hardware
  - User input = Environment
  - Systems Biology = Running programs
  - **Epigenetics = RAM**

Nanopore: Methylation



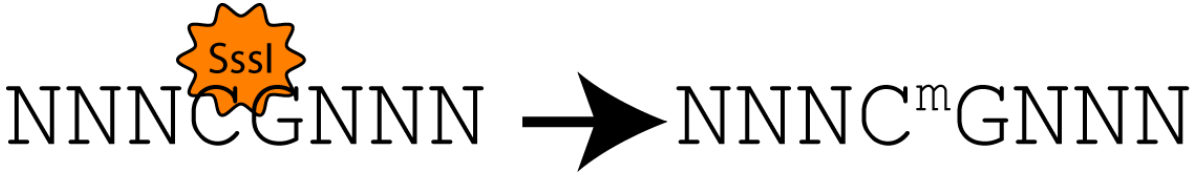
Laszlo, et al. *PNAS* (2013)

- Differences between methylated and unmethylated cytosine have been detected using nanopores.
- Methylation state can be called with 90% accuracy.
- We have writted a methylation detector for Oxford Nanopore for 5-methylcytosine.

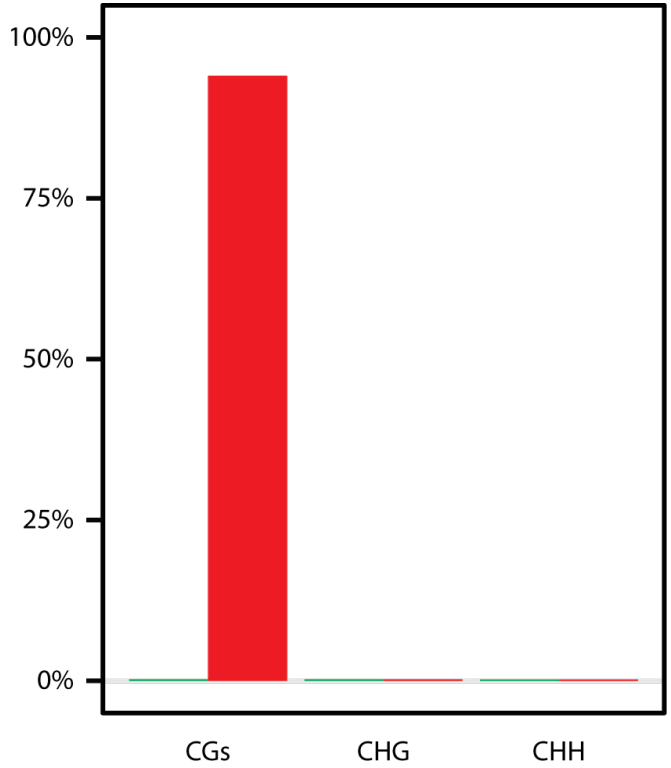


Schreiber, et al. *PNAS.* (2013)

Generation of methylated Samples

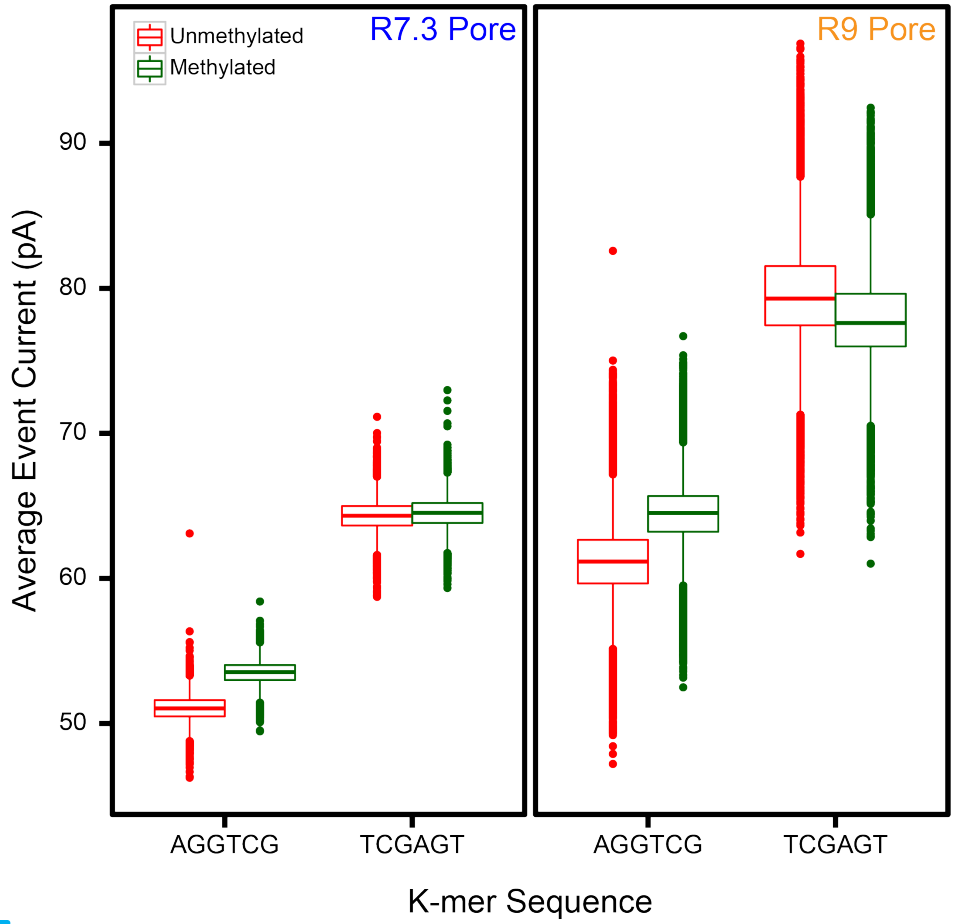


- To generated methylated samples, we treat unmethylated DNA (lambda, dam-/dcm- E. Coli, PCR product) with M. SssI methyltransferase
- We confirm the CG specific methylation using Illumina bisulfite sequencing of the sample – pictured right is methylation in different contexts E. Coli dataset treated with M. SssI (red) versus untreated (green)



Emission Probabilities

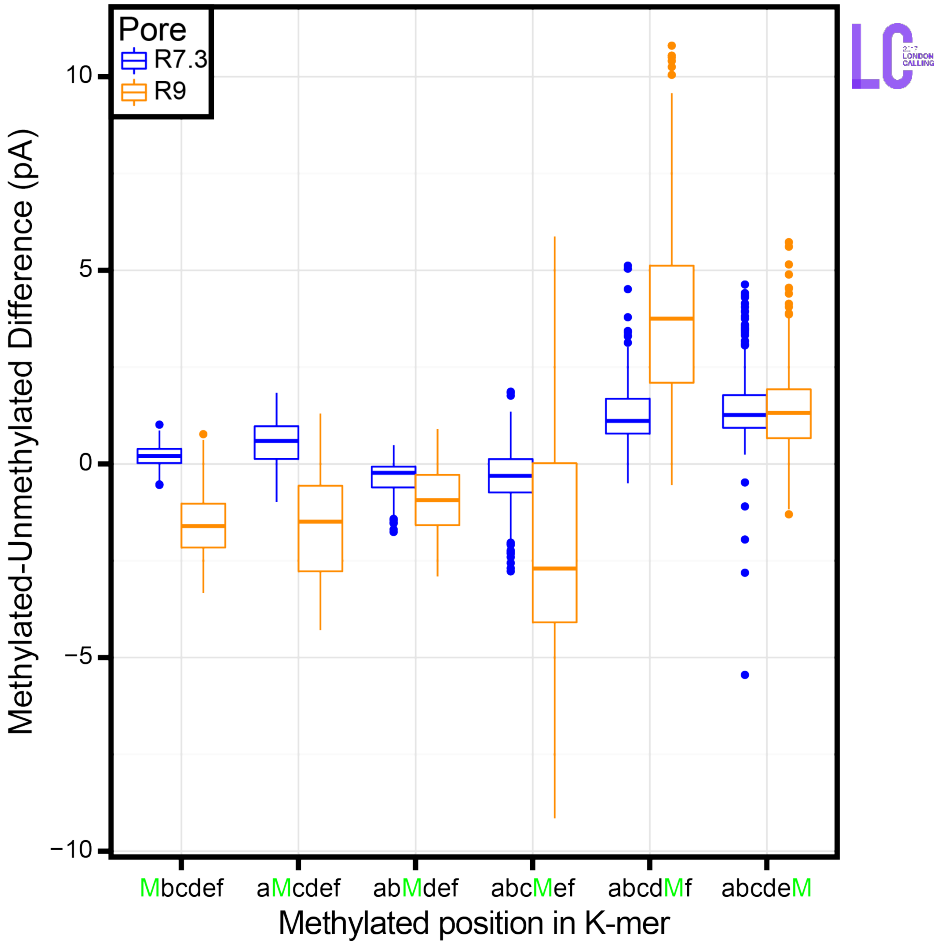
- We measured distributions of current for k-mers from *E. Coli* M.SssI treated (methylated; green) and untreated (unmethylated; red) samples on both R7.3 and R9 flowcells.
- Boxplots of AGGTCG and TCGAGT k-mers which both contain CGs show significant differences in current in some cases (AGGTCG R7.3) and little to none in others (TCGAGT R7.3)
- R9 current distribution seem wider in both cases, but gives better discrimination in TCGAGT.



Simpson, Workman, *Nature Methods* (2017)

Distance of methylation effect

- We looked at the difference in current levels dependent on the position of the methylated base – plotted are the current differences for R7.3(blue) and R9 pores(orange).
- Signal seems again stronger but more variable for R9 pores than R7.3
- Methylation can either reduce current or increase it.
- Some positions are more sensitive to methylation than others.



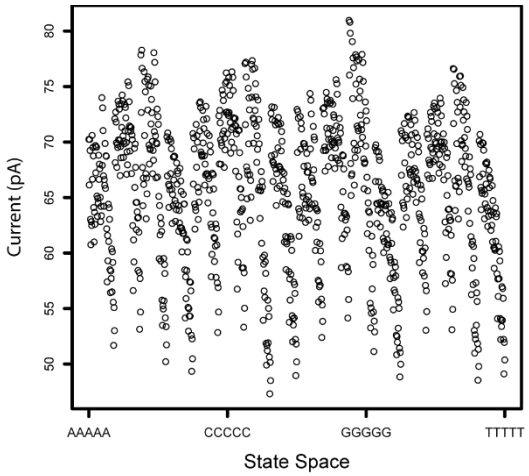
Simpson, Workman, *Nature Methods* (2017)

Nanopore: nanopolish methyltrain

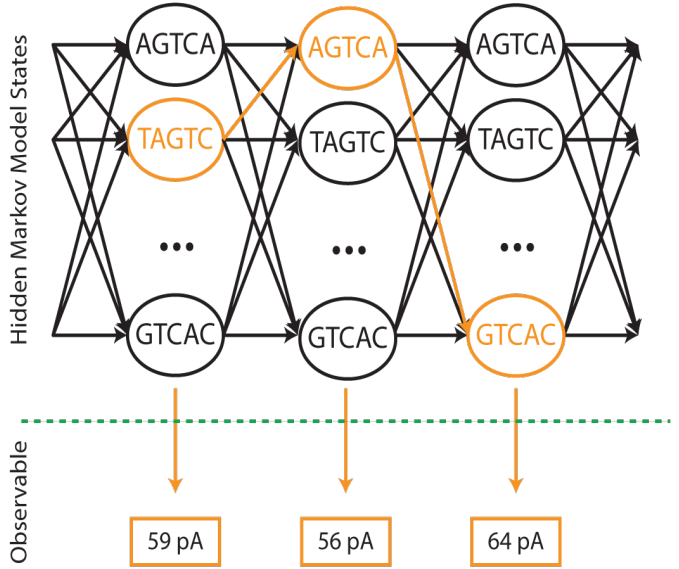
- Multiple bases influence the current passing through the pore.
- Current basecallers use a neural-network based methodology to call bases.
- We currently use a HMM based classifier to call methylation
- With *nanopolish* we can call the probability:

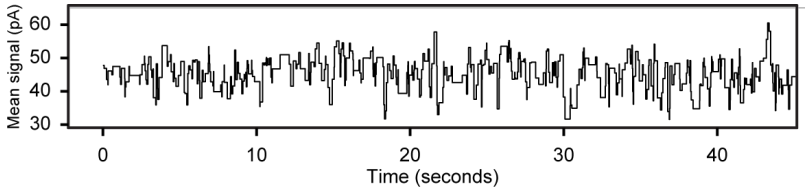
$$\frac{P(\mathcal{D}|S_m)}{P(\mathcal{D}|S_r)}$$

- Where S_m is the probability methylated for a given observable D and S_r the probability unmethylated
- We then take the log of this likelihood ratio.

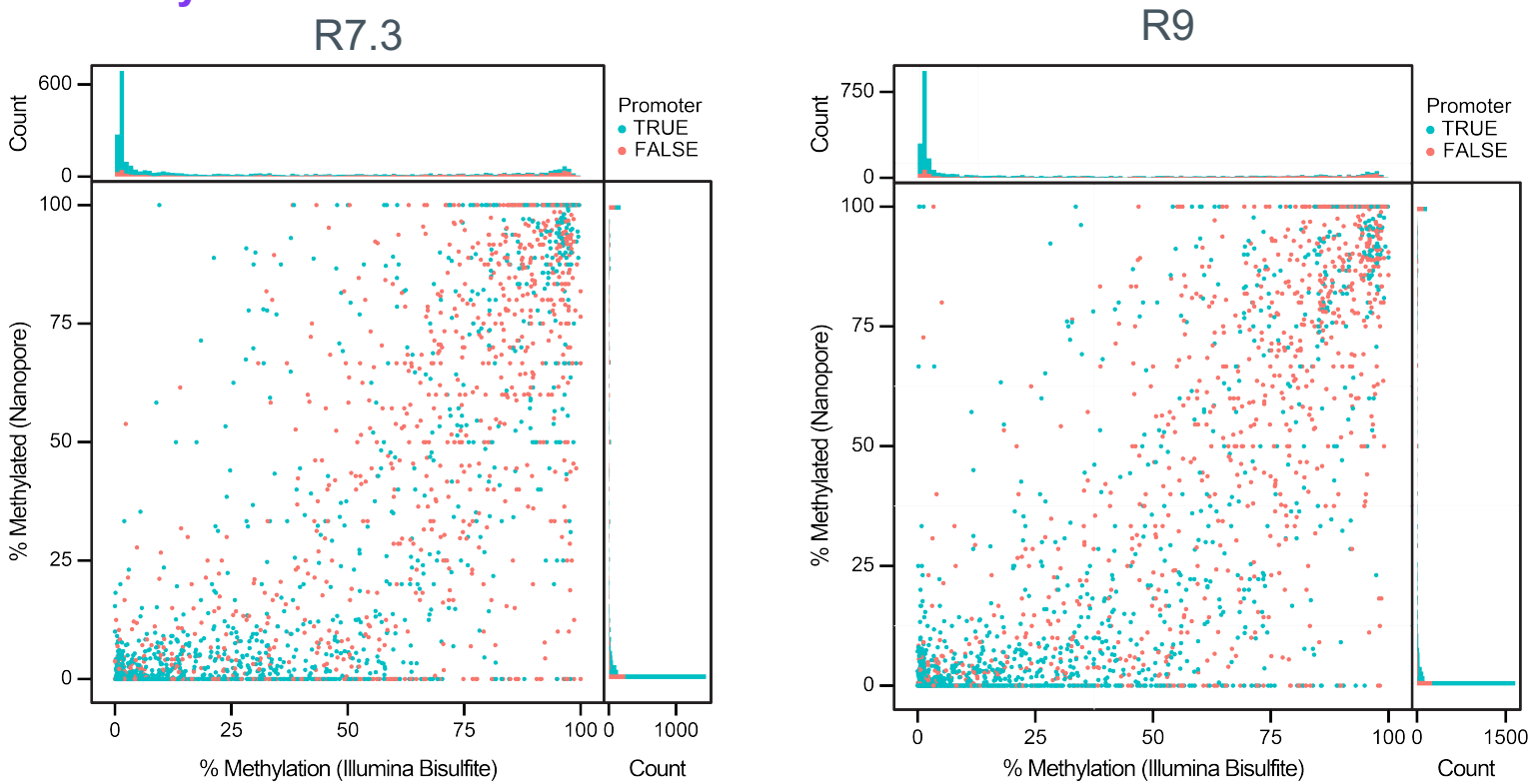


$$\delta_k \prod_t P(I(t)\,|\,k_t) \times T_{(t-1)(t)}$$





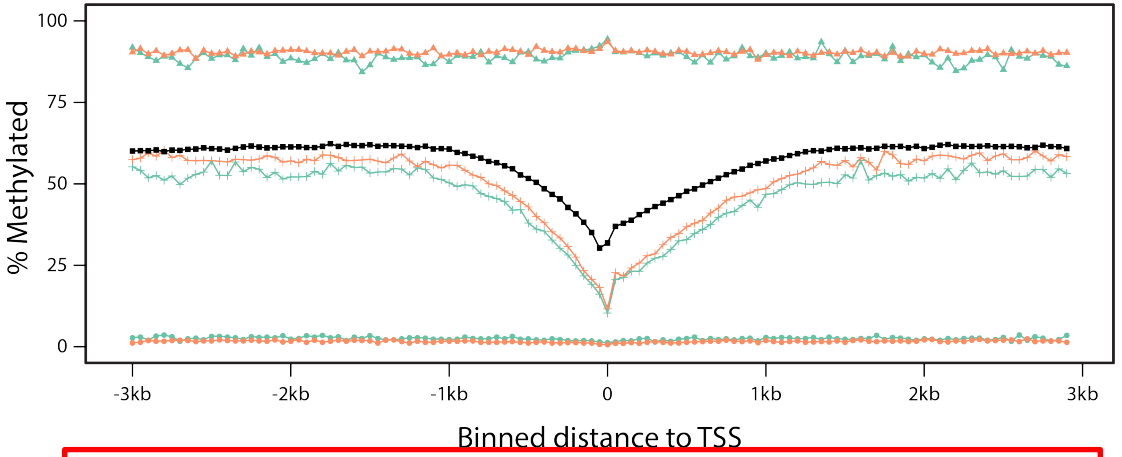
NA12878 Methylation

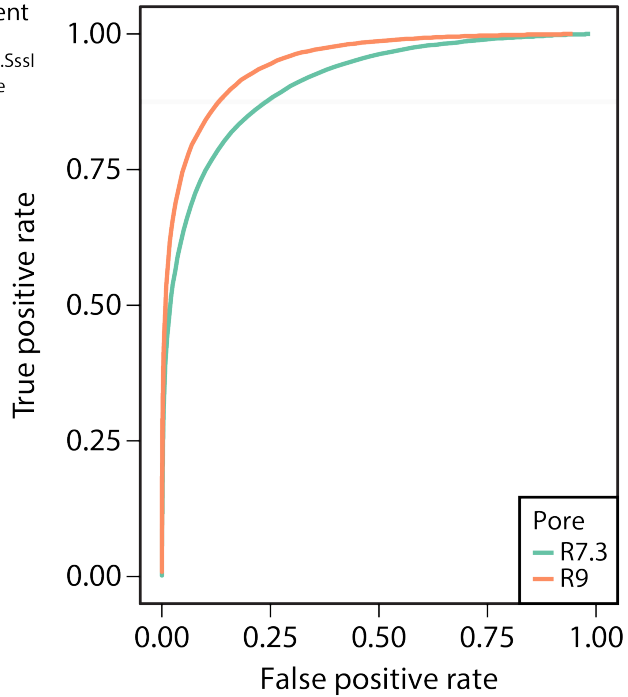


Simpson, Workman, *Nature Methods* (2017)

- NA12878 (lymphoblast) gDNA: Illumina WGBS on X-axis (24X coverage) (SRA: GSM1002650) vs. R7.3 (0.02X) or R9 (0.13X) nanopore sequencing.
- Correlation of 0.83 (R7.3) and 0.84 (R9) – most gene promoters unmethylated

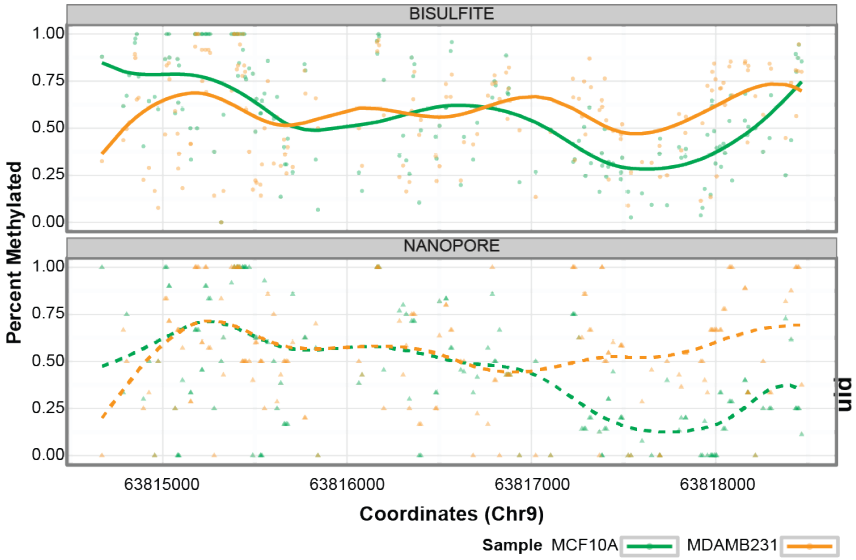
Binned Methylation vs. Transcription Start Sites

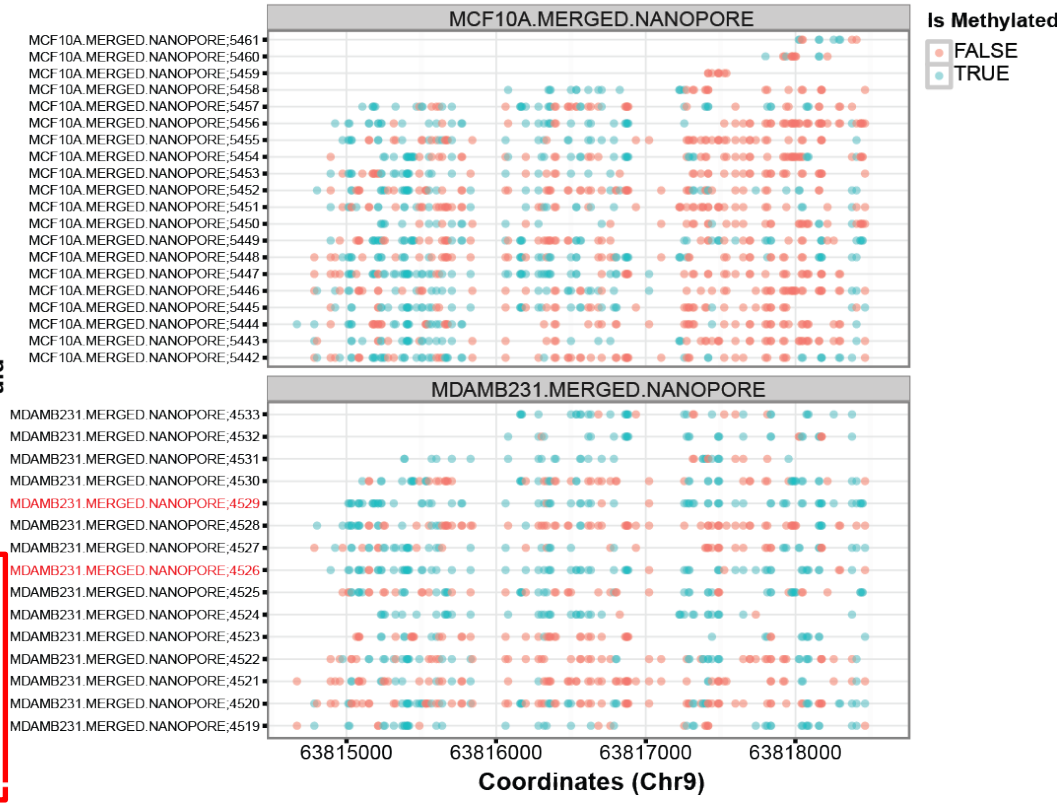




- On human genomic samples:
- Binning methylation levels vs. distance to TSS sites, compared to bisulfite data (NA12878).
- We also generated completely methylated (M.SssI treated; ~95% meth) and unmethylated – used to generate the ROC curve (right)
- R7.3 91% accurate at 68% of sites
- R9 94% accurate at 77% of sites

Cancer-Normal Comparison



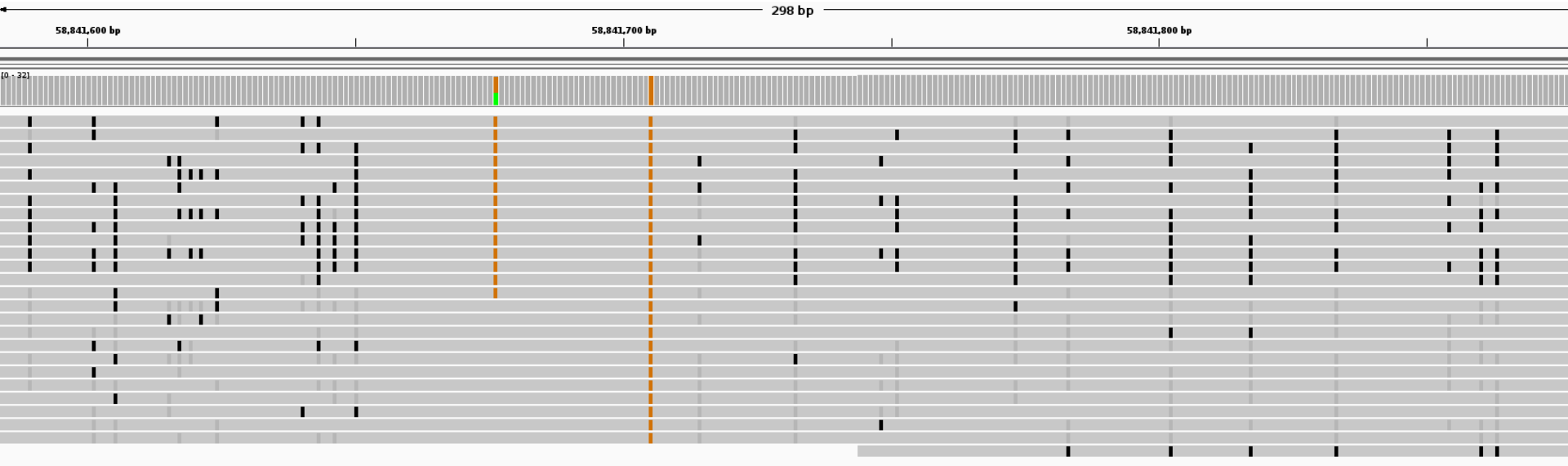


- Reduced representation method:12.5Mb of the genome (3.5-6kb size selection)
- We sequenced this fraction on nanopore and bisulfite Illumina seq
- Long reads measure *phased* methylation

Simpson, Workman, in revision (2016)

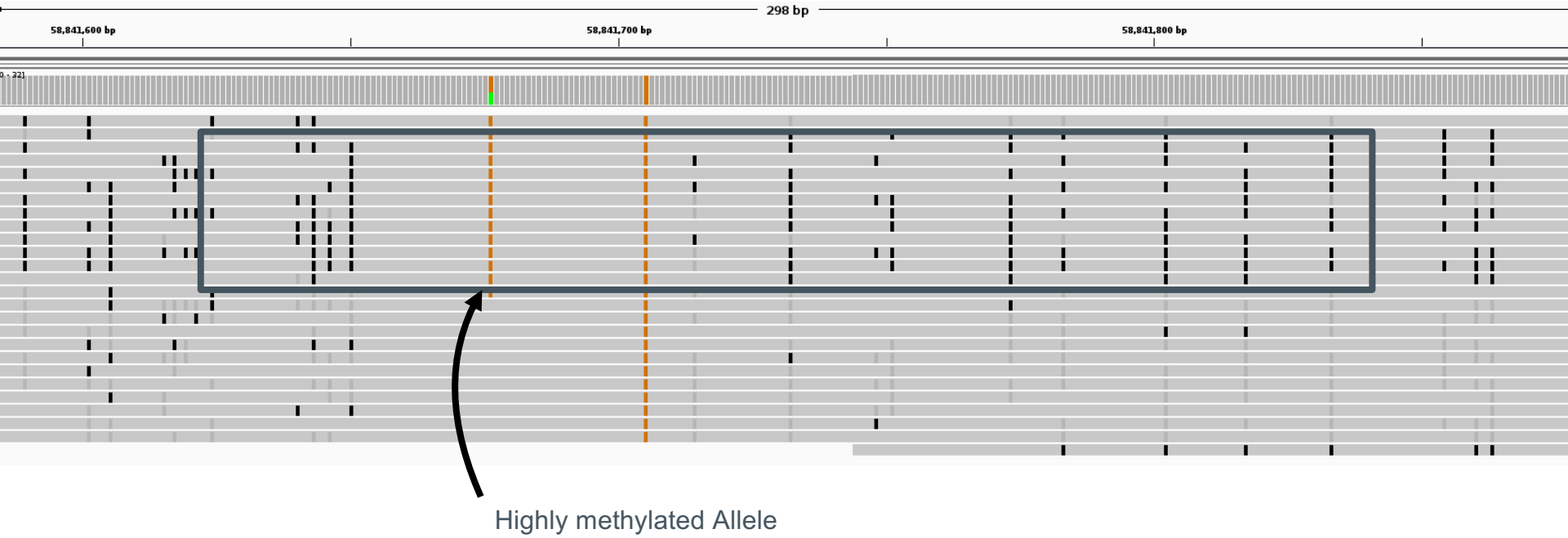
Haplotype-Phased Methylation

nanopolish has experimental support for phasing methylation patterns



Haplotype-Phased Methylation

Nanopolish has experimental support for phasing methylation patterns. (From NA12878 data)



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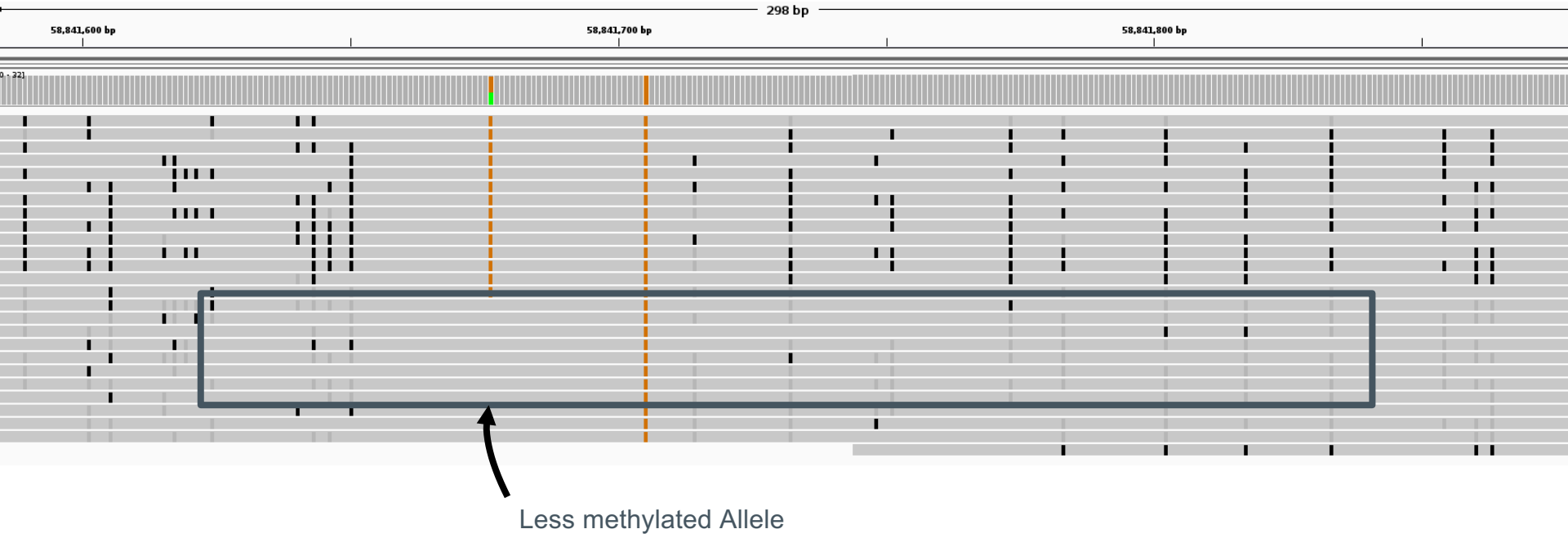
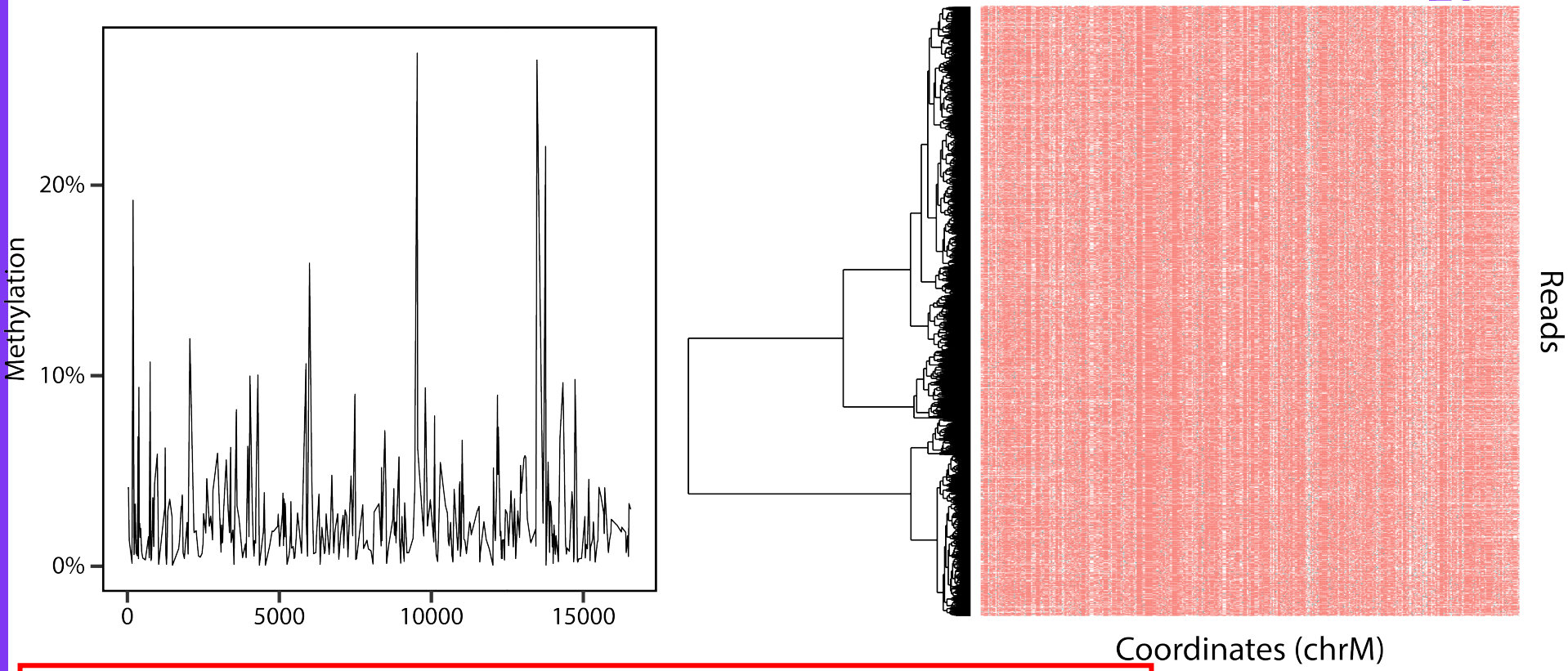


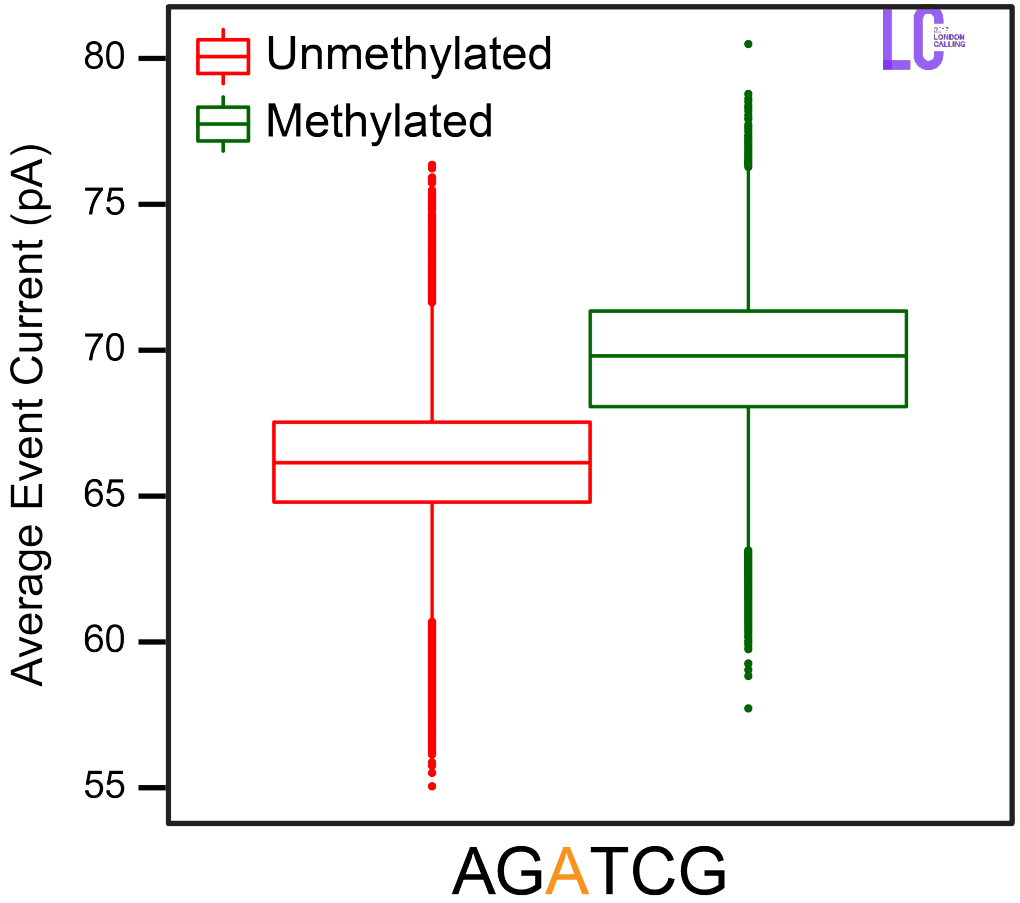
Mitochondrial methylation/clustering



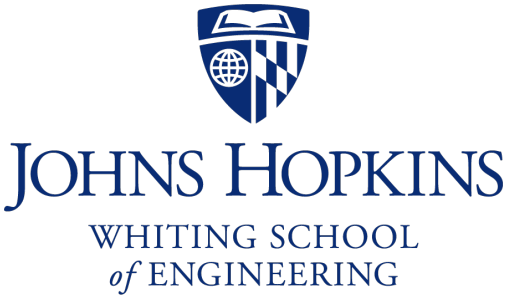
Preliminary data clustering mitochondrial CG methylation from MCF10A

Future Work

- Expand to non-CpG methylation
- Expand to non 5-methylcytosine methylation
  - Strong signal for N6-methyladenine
- Apply to clinical samples
- Exogenous labeling of DNA and readout



Acknowledgments



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