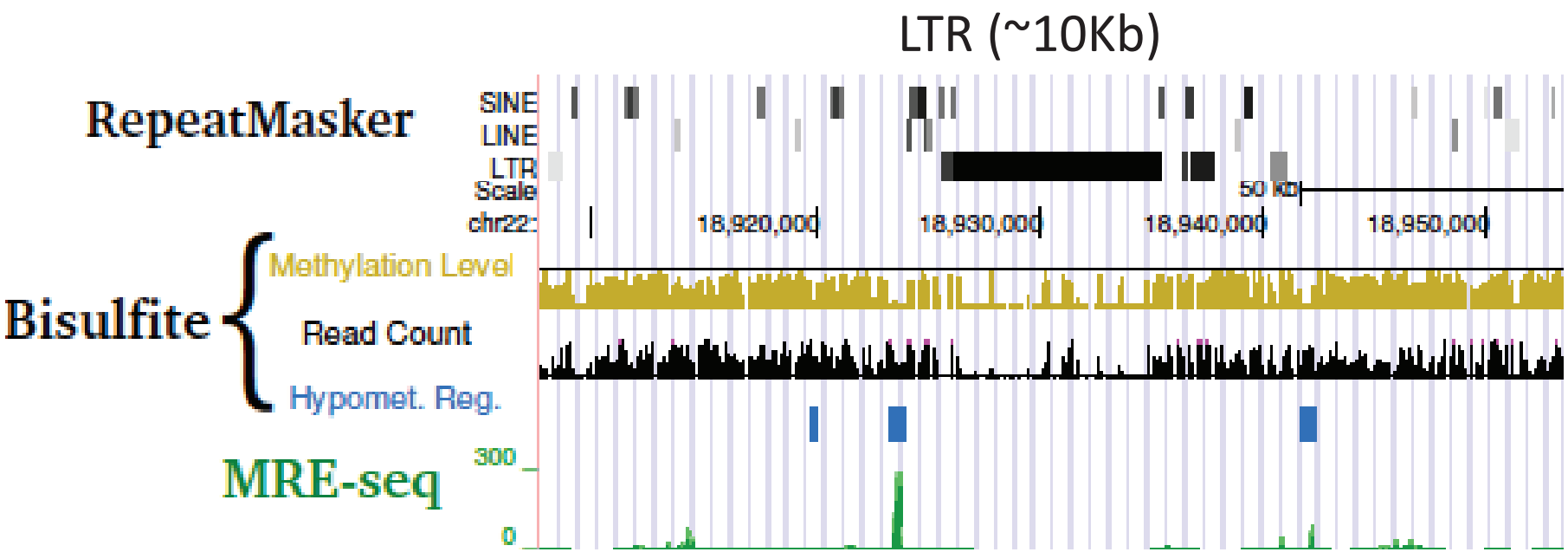
Understanding methylome, metagenome, structural variants using SMRT sequencing

Shinichi Morishita
University of Tokyo

Can we characterize methylome in repeats?

Bisulfite-treatment often divides DNA into ~600bp fragments.

We extended SMRT sequencing.



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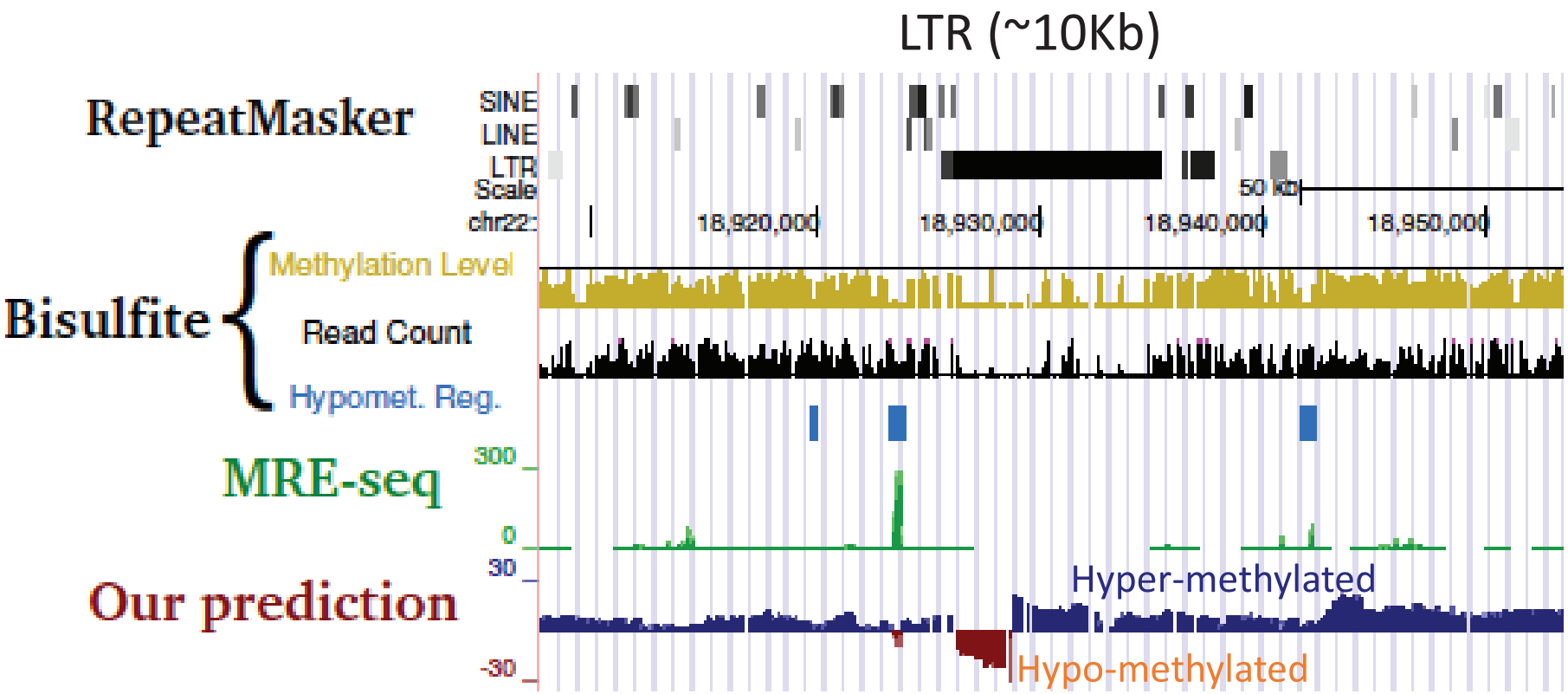

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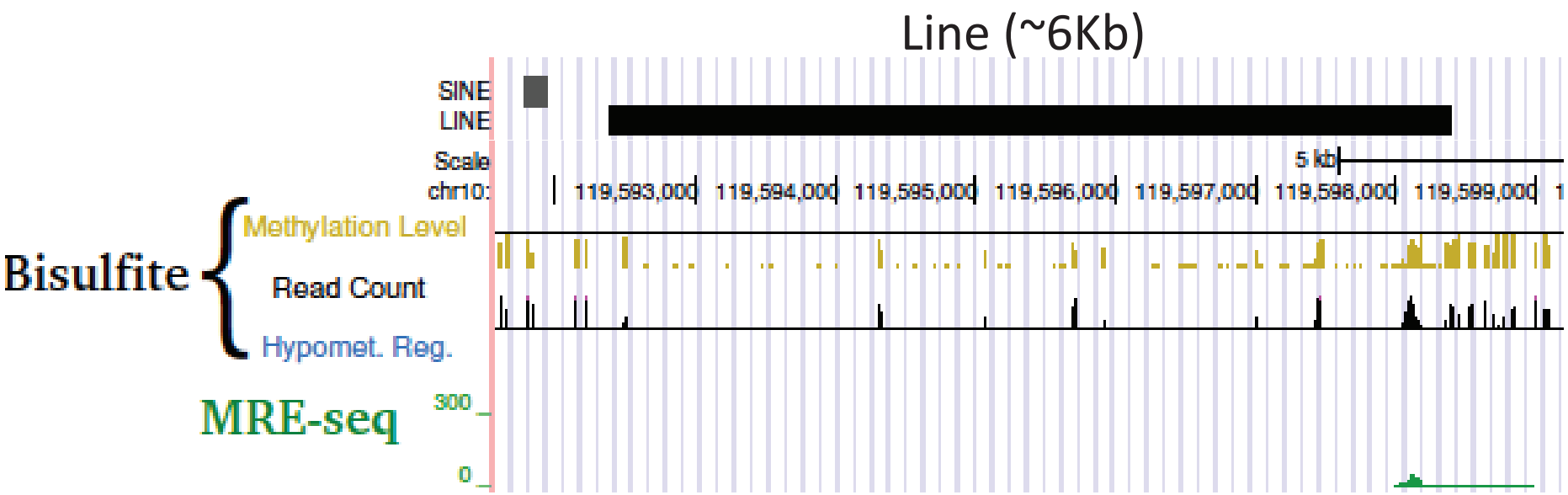

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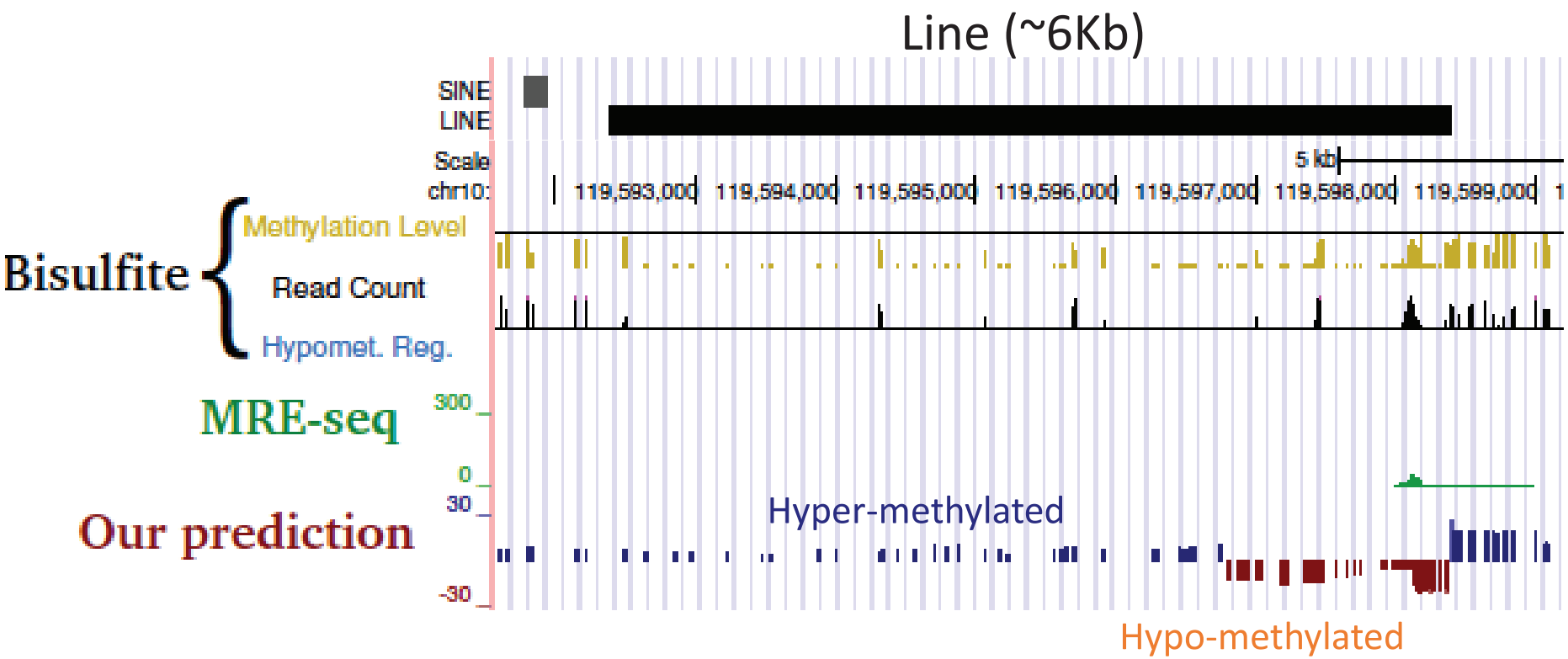

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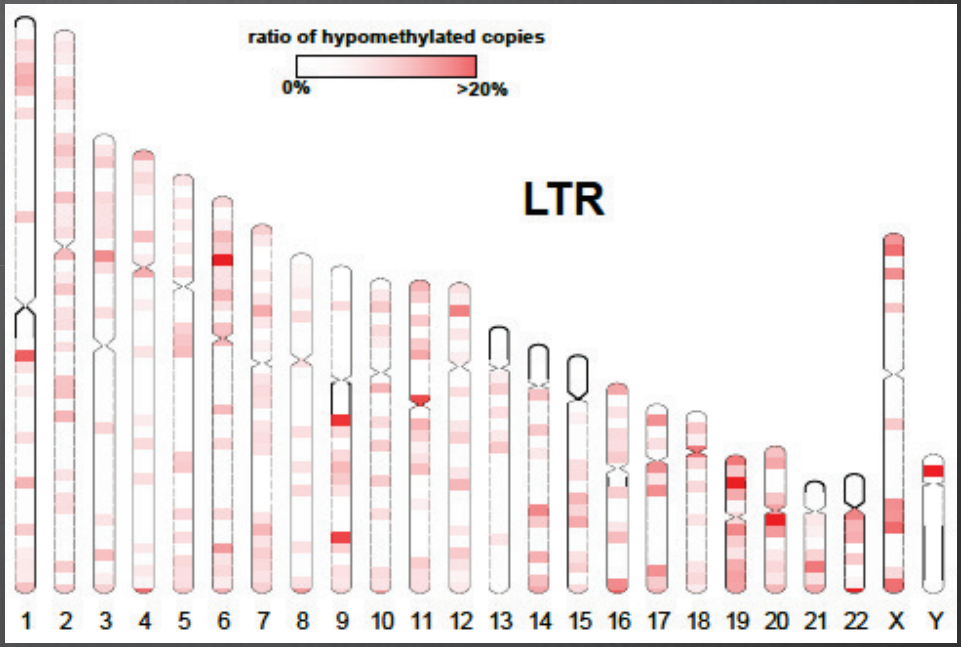
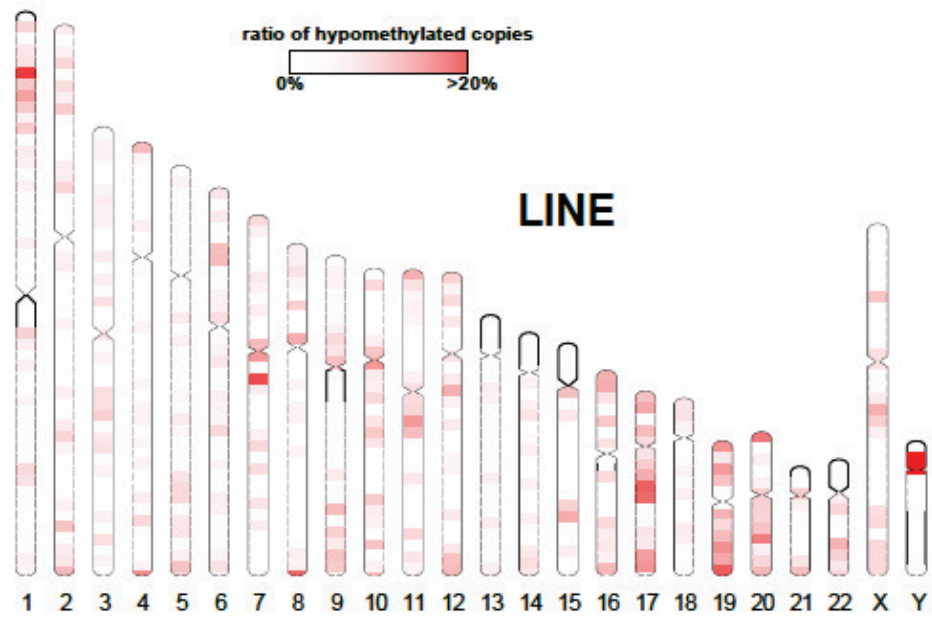
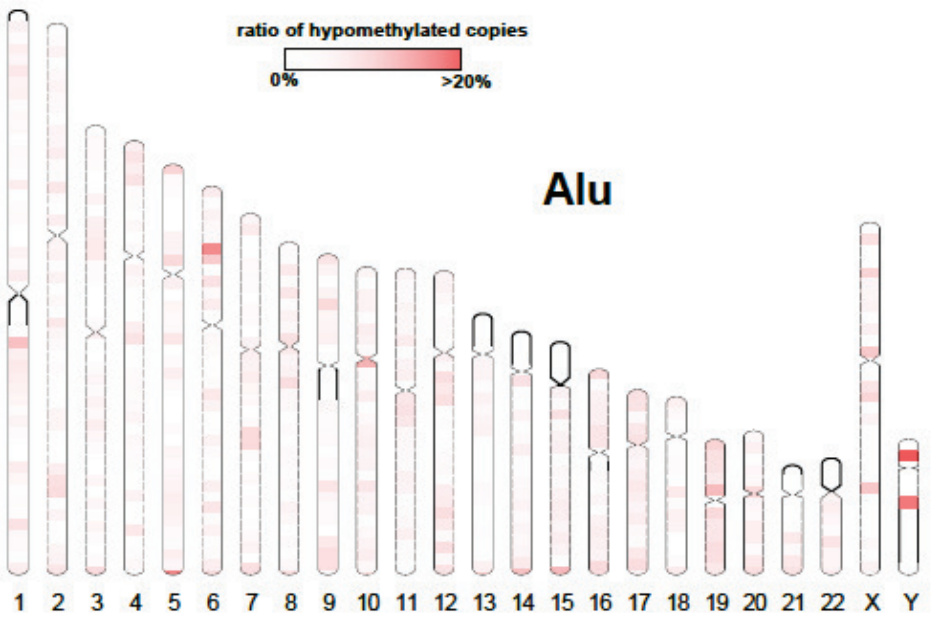
Landscape of hypomethylated repeats in the human genome







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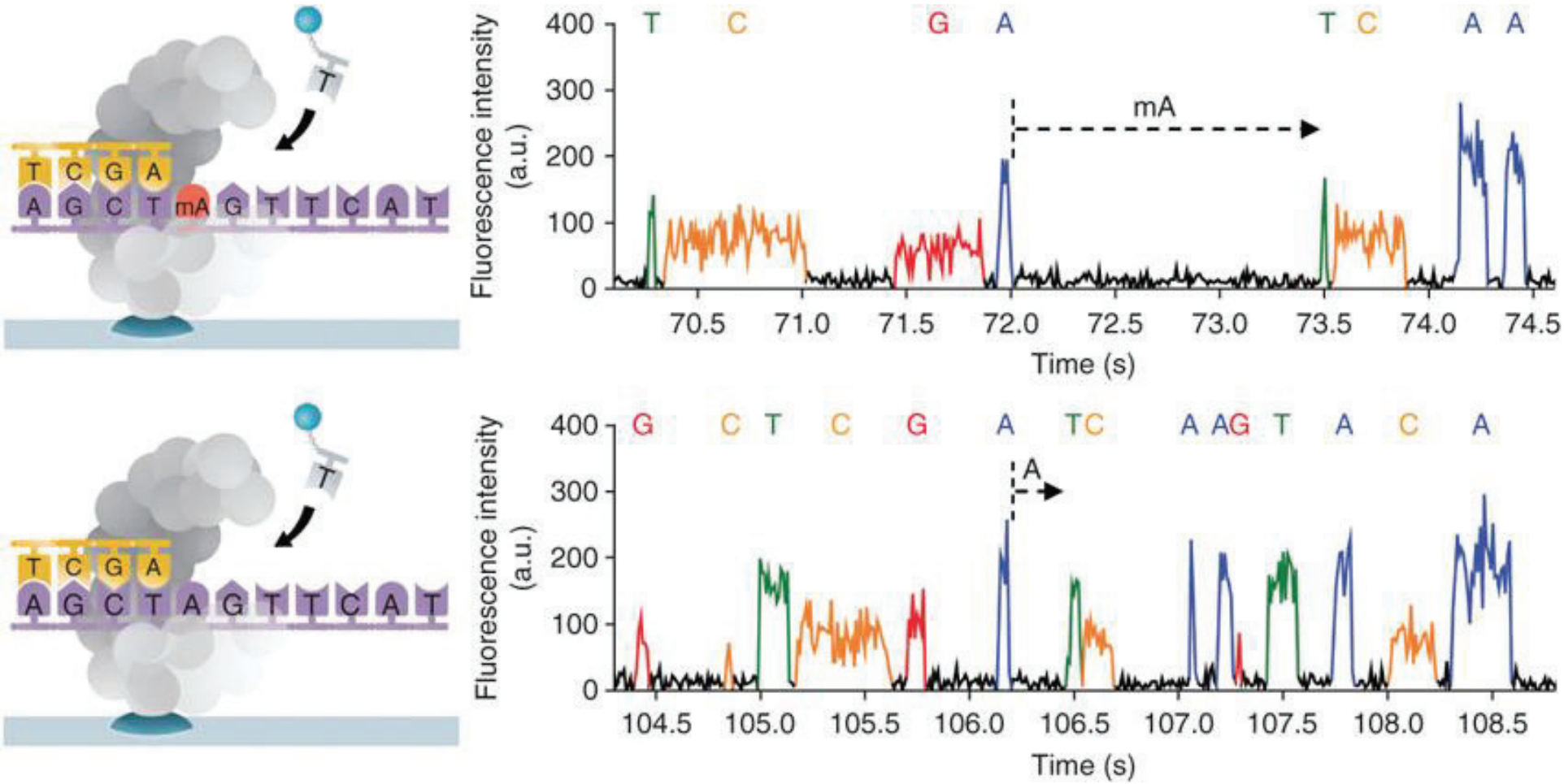
Estimating CpG methylation using SMRT sequencing



Benjamin A Flusberg, Dale R Webster, Jessica H Lee, Kevin J Travers, Eric C Olivares, Tyson A Clark, Jonas Korlach & Stephen W Turner. Direct detection of DNA methylation during single-molecule, real-time sequencing. Nature Methods 7, 461 - 465 (2010)

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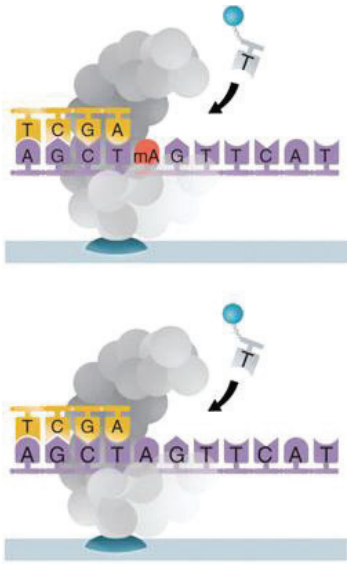
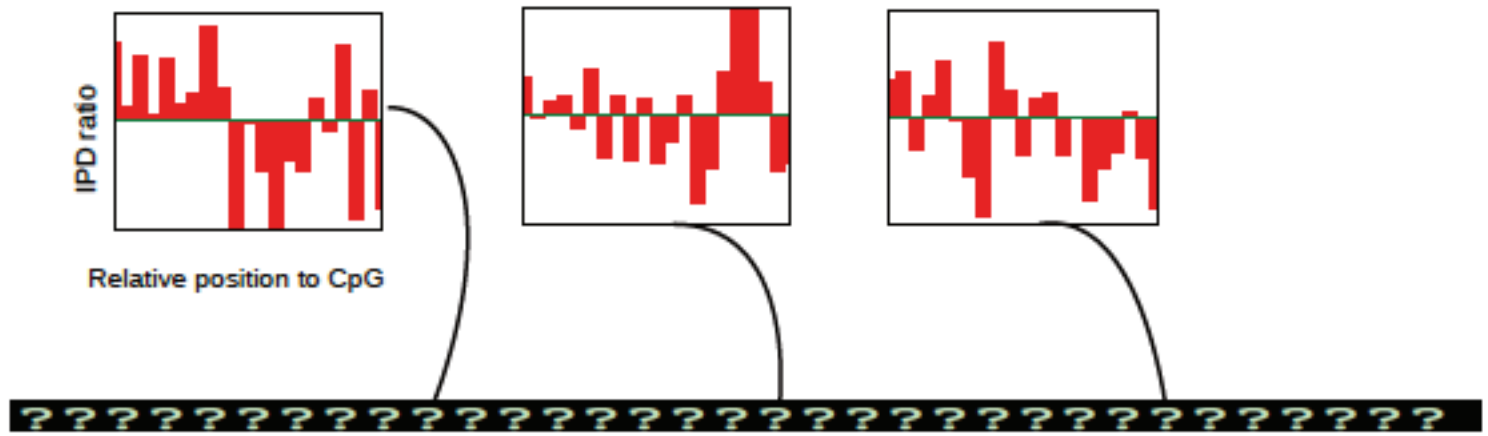


Subtle signals around CpGs…



CpG sites

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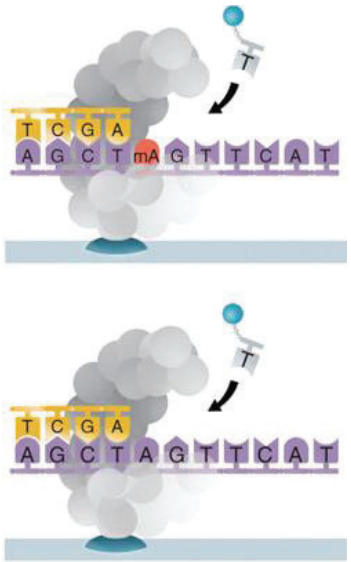
Estimating CpG methylation using SMRT sequencing



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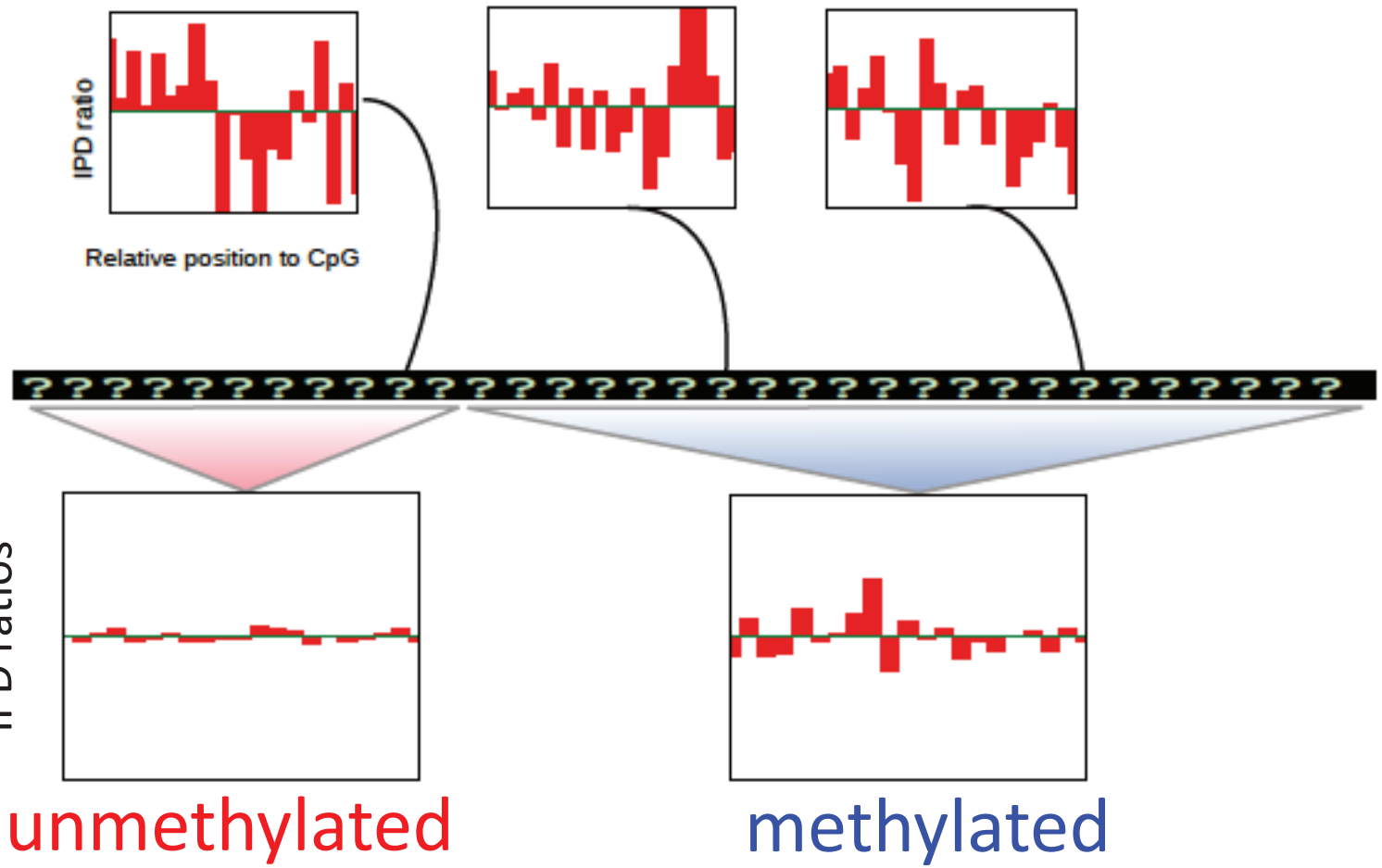

A linear-time optimization algorithm (2.265 s to process 1 Mbp on an Intel i7-3612QM processor)

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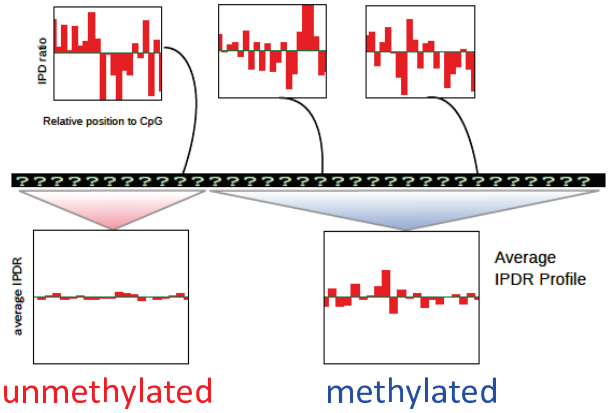
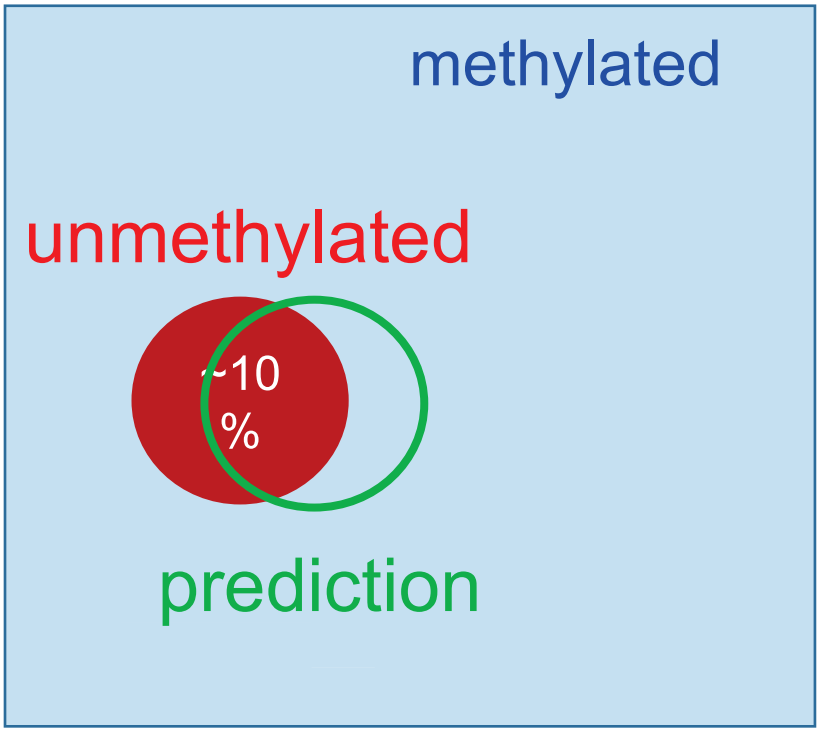
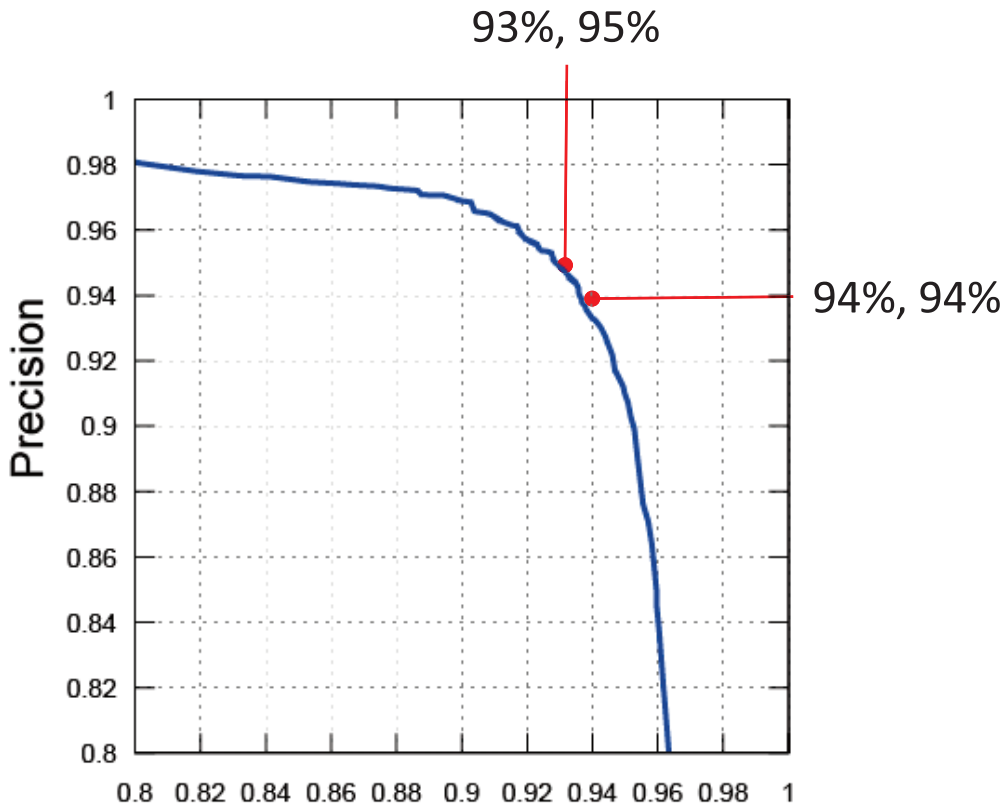




Bisulfite sequencing



minimum window size = 35bp
~20-fold SMRT subread coverage

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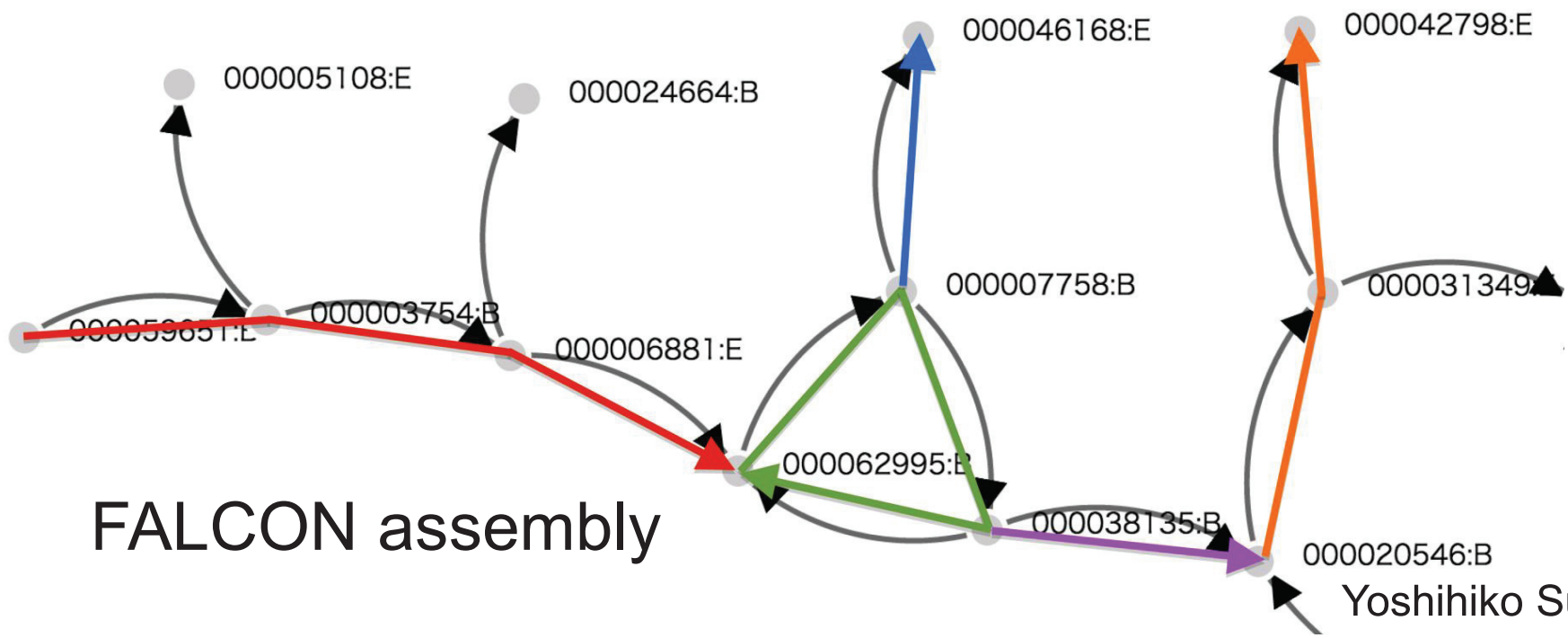
SMRT sequencing of un-isolated bacteria in flora

How much of bacterial genomes are unknown?

- Used lysozyme instead of QIAamp DNA Stool Mini Kit
  (Morita et al. Microbes and Environments, 2007)
- Avg. subread length = 7-9Kb, N50 = 13-15Kb (4 samples)
- 61 contigs of >1Mb in size, 67% are unknown

Assembly considering abundance of organisms



FALCON assembly

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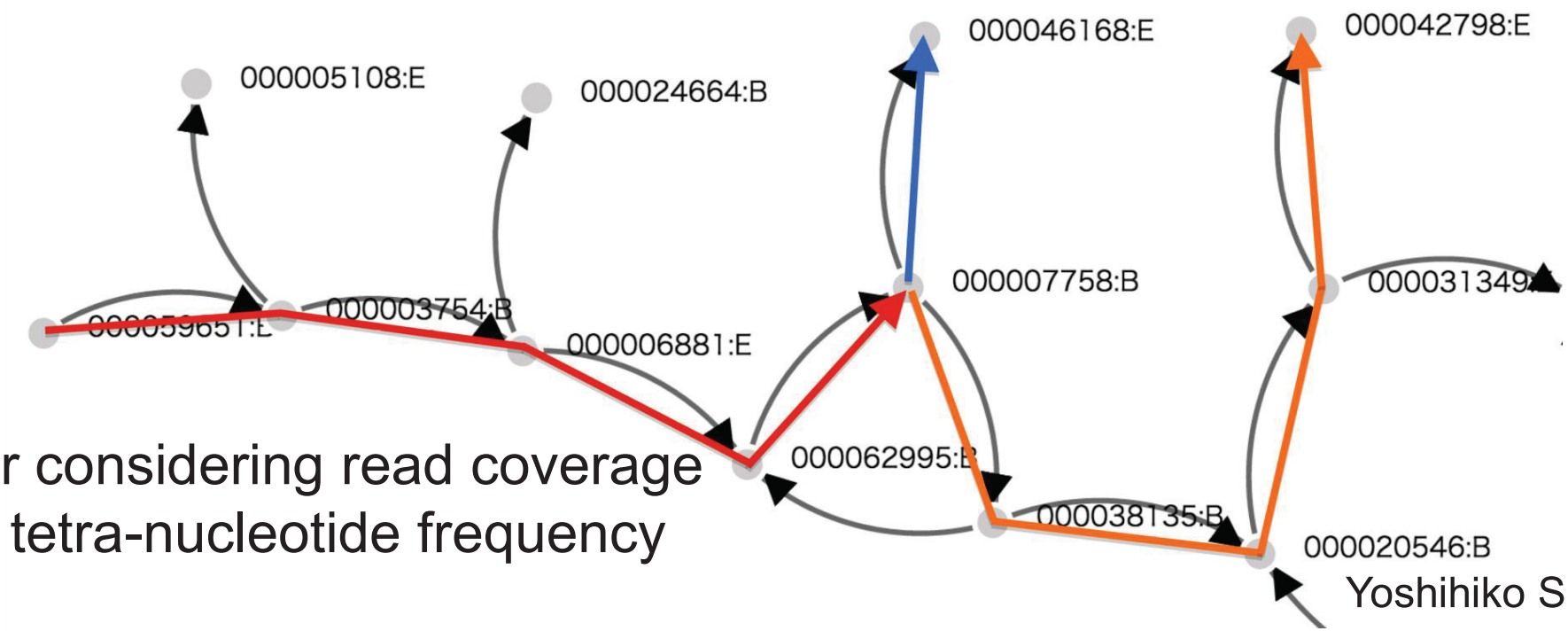

After considering read coverage and tetra-nucleotide frequency

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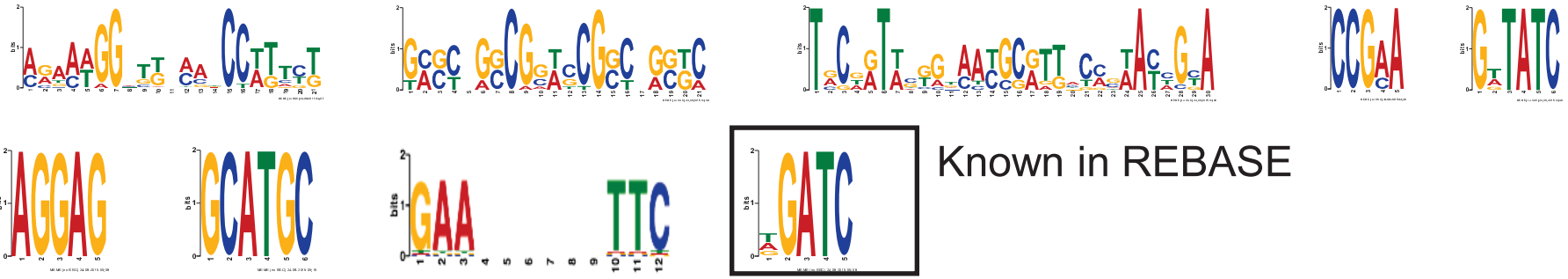
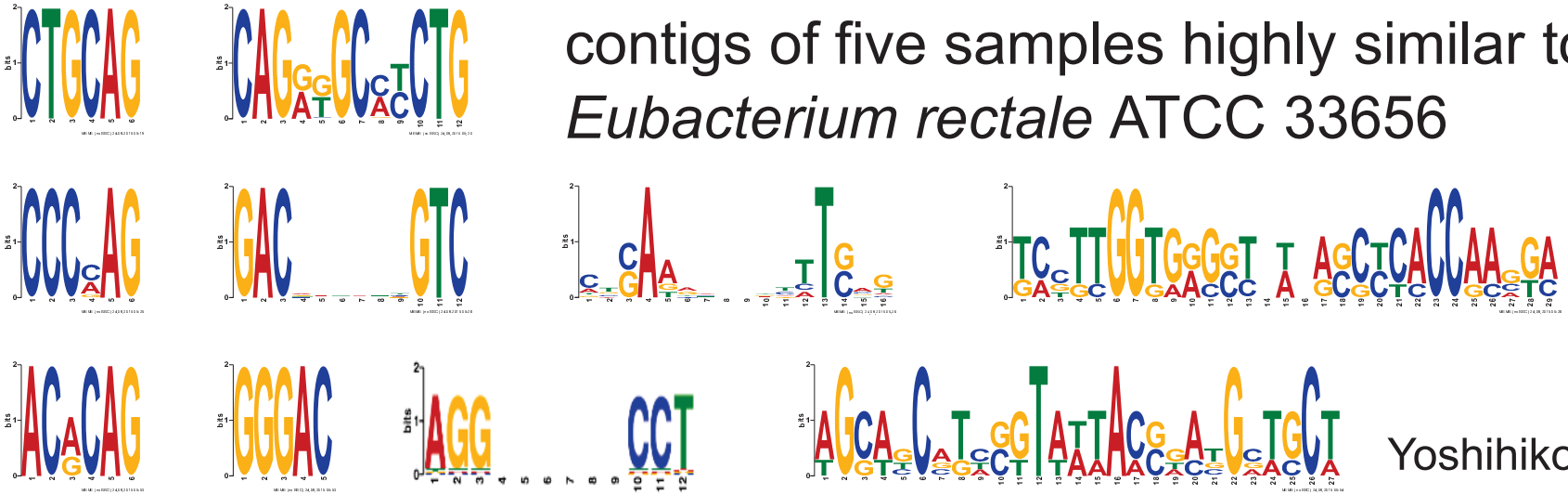
SMRT sequencing of un-isolated bacteria in flora

Might DNA modification patterns be different between un-isolated and isolated bacteria?



Known in REBASE

Motifs with candidate DNA modifications in contigs of five samples highly similar to *Eubacterium rectale* ATCC 33656



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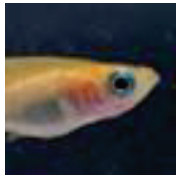
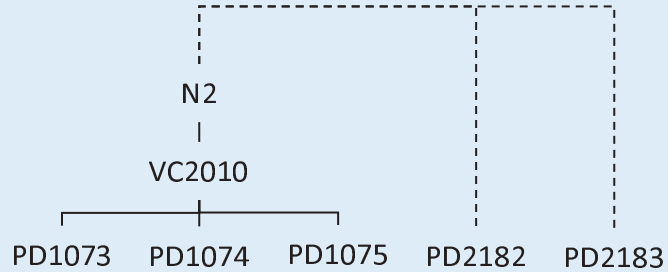
Can we correct deficits in reference genomes using SMRT sequencing?

x 3

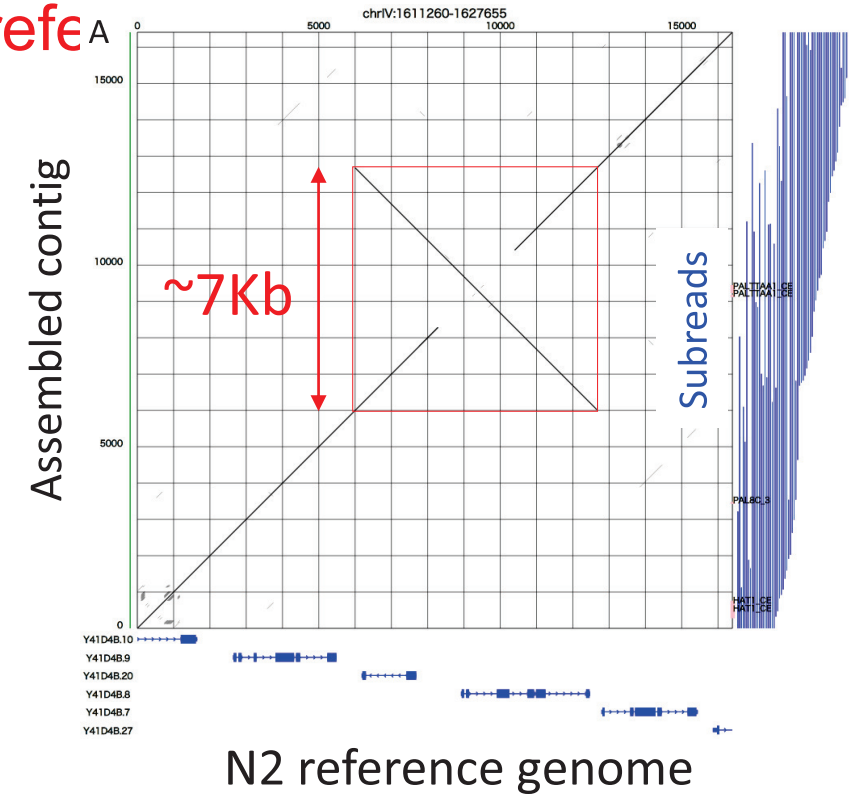


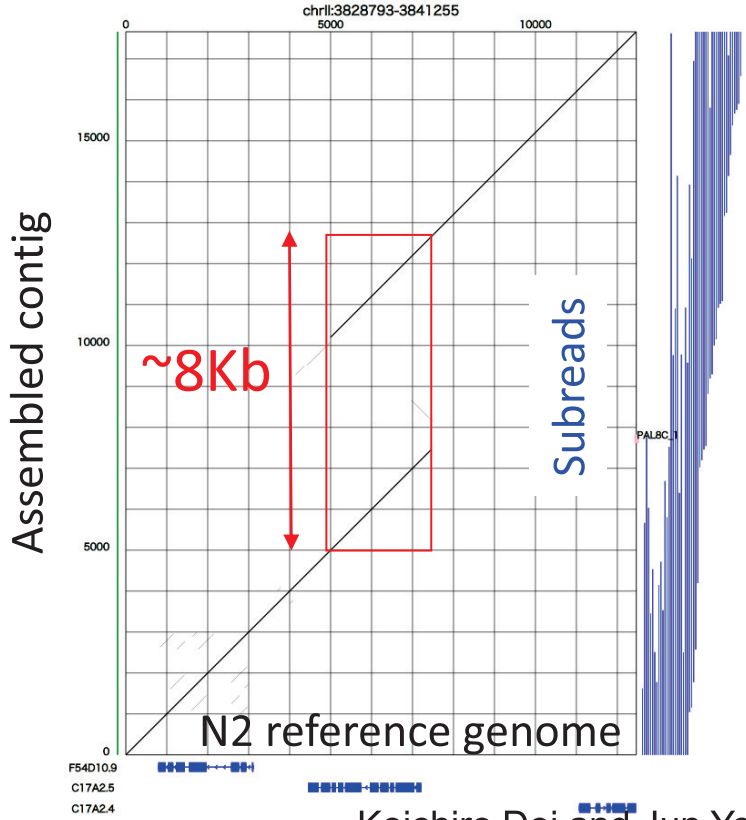
x 3

x 5



Detected 30 duplications and 24 inversions absent in the worm refe





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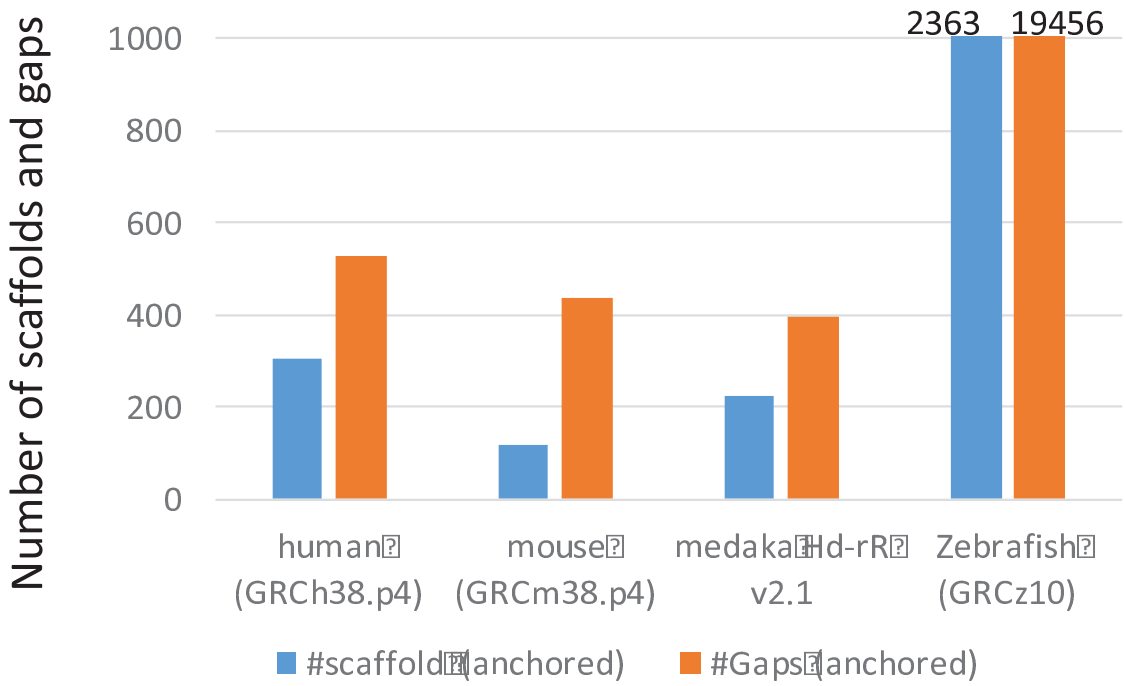
Can we correct deficits in reference genomes using SMRT sequencing?

High concordance of PacBio contigs with other information (medaka)

2,510 genetic markers (only 4% contigs had deficits)

25,148 BAC ends (only 0.04% are inconsistent)

~90% of contigs on chromosomes (N50 contig = ~2.5Mb)



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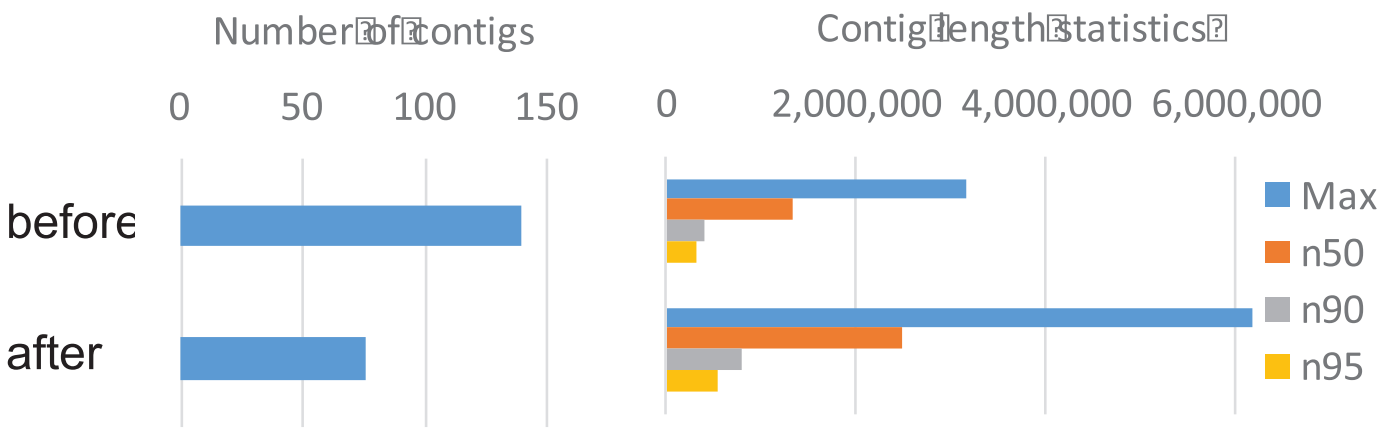
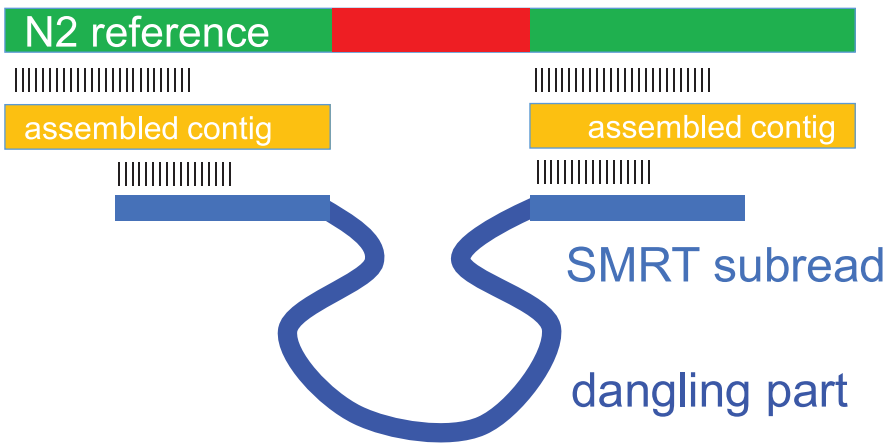
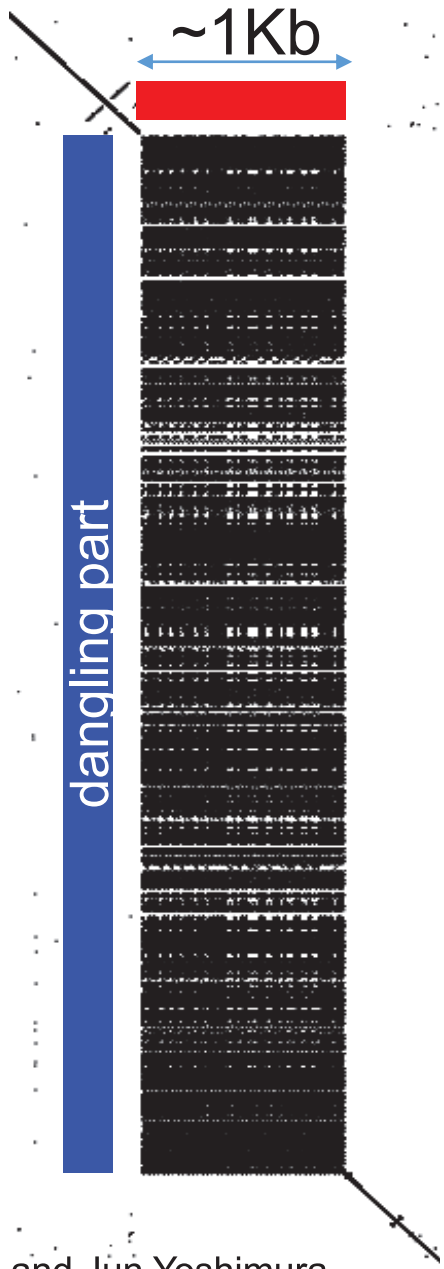
Filling gaps in the worm assembly using PBJelly







Koichiro Doi and Jun Yoshimura

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University of Tokyo	
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