Oxford Nanopore Technologies

# Performance of Oxford Nanopore whole-genome methylation sequencing in human genetics applications

Genomic DNA can be sequenced on Oxford Nanopore devices without the need for fragmentation, amplification, or strand synthesis, improving mappability and retaining long-range data for methylation phasing

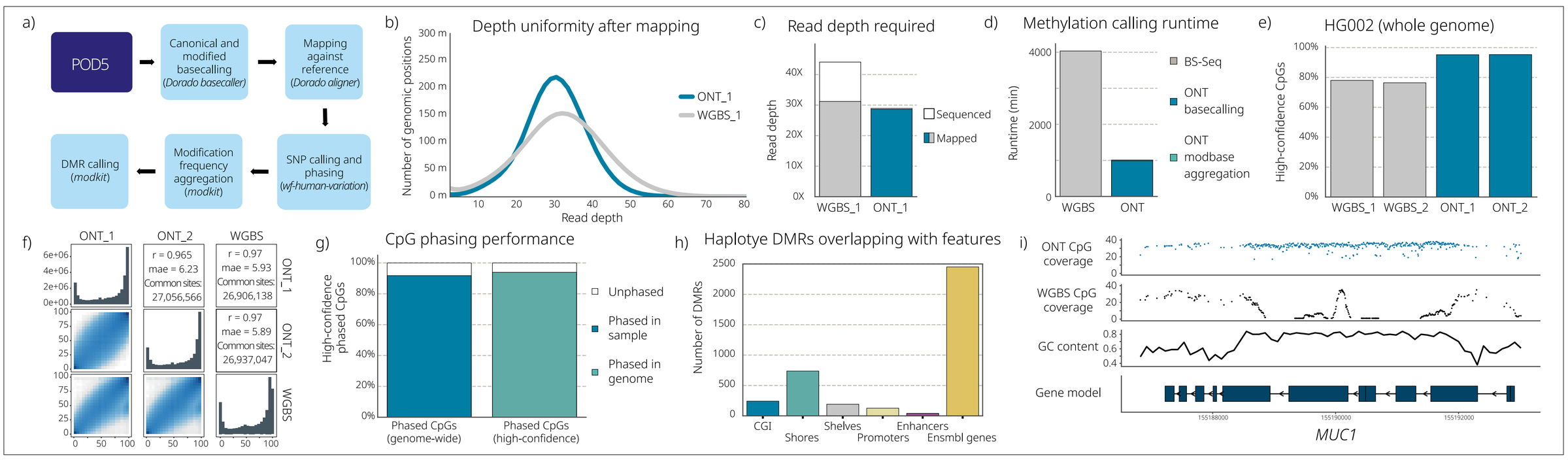


**Fig. 1** Benchmarking Dorado methylation calling. a) pipeline, b) evenness, c) depth, d) runtime, e) called CpGs, f) reproducibility, g) phasing, h) haplotype DMR annotation, and i) Coverage in a low mapability zone.

## Benchmarking of nanopore methylation analysis reveals lower bias, higher mapping rates, greater reproducibility, and faster analysis than seen with bisulfite data

Modification of cytosine to 5-methylcytosine (5mC) is the most common epigenetic marker in mammalian DNA, typically occurring in a CpG dinucleotide context. This modification can alter gene expression by suppressing transcription initiation. During Oxford Nanopore sequencing of native DNA, the signature of modified bases is present in the raw signal, and can be decoded alongside the canonical base sequence. To benchmark Oxford Nanopore methylation calling using Dorado (HAC v5.2.0 5mCG_5hmCG model), we generated two replicates of the HG002 human genome reference sample, obtaining 30x coverage per replicate (ONT_1, ONT_2). We analysed all samples using the pipeline shown (**Fig. 1a**) and compared calls to public short-read whole-genome bisulfite (WGBS) datasets. Nanopore read depth is more uniform than the WGBS data (**Fig. 1b**), maps to the genome fully (**Fig. 1c**) and requires far less analysis time (**Fig. 1d**). We obtain a higher percentage of successfully called CpGs (>90%) at moderate overall read depth (**Fig. 1e**). Oxford Nanopore 5mC calls correlate well with WGBS (**Fig. 1f**). Using the EPI2ME™ wf-human-variation workflow to call variants and phase reads, we were able to phase methylation calls across >90% of the called high-confidence CpGs and >90% of the CpGs in the human genome (**Fig. 1g**). We were also able to identify features that are differentially methylated between haplotypes (**Fig. 1h**). To explore methylation calling in regions of low mappability for short reads, we plotted CpG coverage and GC content over the *MUC1* locus (**Fig. 1i**).

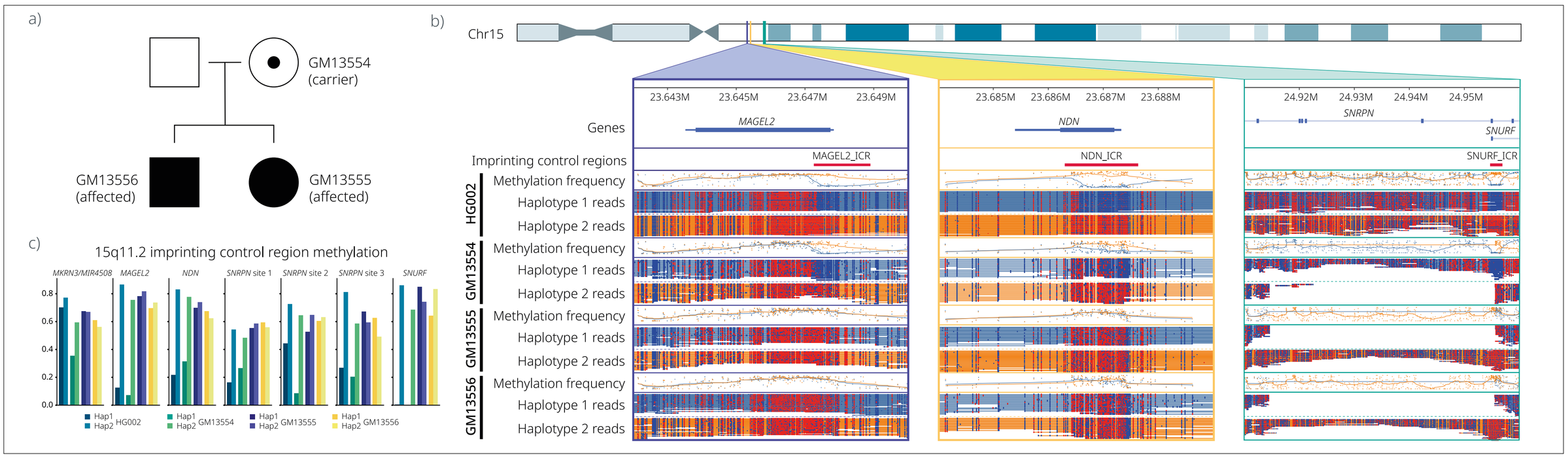


**Fig. 2** Methylation in a Prader-Willi syndrome family. a) pedigree, b) imprinting control region (ICR) deletion (right) and methylation calls over three ICRs, and c) mean methylation per haplotype in 15q11.2 ICRs.

## Paternally-inherited partial deletion of *SNURF* imprinting control region disrupts imprinting across Prader-Willi locus (15q11.2) in research samples from a family affected by Prader-Willi syndrome

Prader-Willi syndrome is a genomic imprinting disorder caused by loss of paternal gene expression across the chromosome 15q11.2-q13 locus. One mechanism is the deletion or partial deletion of an imprinting control region (ICR) overlapping *SNRPN* exon alpha / *SNURF* exon 1. This genomic lesion is known to disrupt parent-of-origin-specific methylation patterning throughout the larger locus when inherited paternally. To demonstrate the utility of Oxford Nanopore whole-genome methylation sequencing in resolving such phenomona, we sequenced a previously characterised family group available through the Coriell cell line repository (family #1618; **Fig. 2a**) and compared the data to the GIAB HG002 cell line. We confirmed a ~40 kb deletion which removes all of *SNURF* exon 1 and about half of the corresponding ICR, present in both the unaffected father and both affected children (**Fig 2b**, right). The father's methylation patterning is consistent with maternal inheritance of the deletion, explaining his unaffected status. Overall, seven genic ICRs are known to occur on the 15q11.2 locus, all which follow the same pattern of maternal hypermethylation and paternal hypomethylation (examples in **Fig 2b**, left and centre). SNV calling and phasing with the EPI2ME wf-human-variation pipeline and subsequent haplotype-specific methylation aggregation revealed expected methylation patterns in HG002 and the father, but hypermethylation in both alleles in the affected children (**Fig 2b**, **Fig 2c**).




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