

Reduced-Representation Methylation Sequencing (RRMS) captures 100% of CpG islands and more

The method allows targeting of important regions in the genome, enabling the genome-wide characterisation of methylation patterns across samples of interest in a cost-effective way

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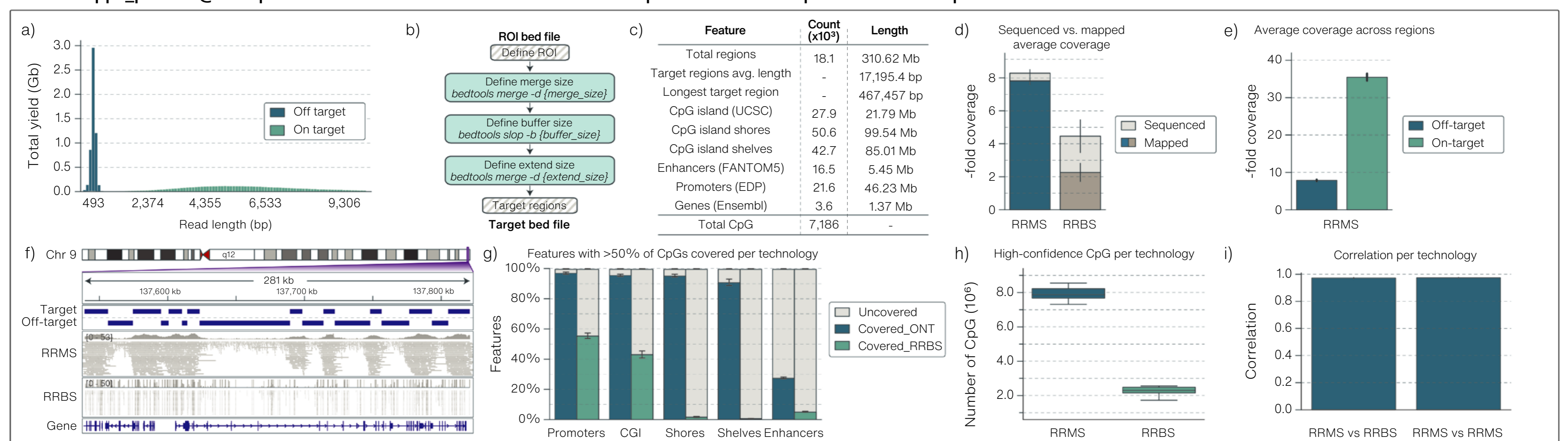


Fig. 1 RRMS using AS a) read length distribution b) bed workflow c) features d) sequenced vs mapped e) coverage f) example region g) overlap with RRBS h) CpGs called i) correlation with RRBS

Adaptive sampling increases coverage of target reads, enabling 8M high-confidence CpGs in key regions of the genome to be called with high accuracy using only a MinION™ Flow Cell

Nanopore sequencing enables direct detection of methylated cytosines (e.g. at CpG sites), without the need for bisulfite conversion. CpG sites frequently occur in high density clusters called CpG islands (CGI) and >60% of human genes have their promoters embedded within CGIs. Changes in methylation patterns within promoters is associated with changes in gene expression. Reduced representation bisulfite sequencing (RRBS) is a method used to obtain genome-wide methylation analysis without the need to sequence the whole genome, but is expensive and time consuming. Furthermore, the complex library preparation method is imprecise and does not specifically target any promoter region. Adaptive sampling (AS) offers a fast, flexible and precise method to enrich for regions of interest (e.g. CGIs) by depleting off-target regions during sequencing itself (Fig. 1a), with no requirement for upfront sample manipulation. Here we combine Oxford Nanopore's methylation detection with AS and compare the performance with RRBS, using two tumour/normal cell line pairs. Our file of AS target regions is prepared using the workflow shown (Fig. 1b) and covers 310 Mb of the genome, including ~28,000 CpG islands and other key features (Fig. 1c). AS retains a higher proportion of data than RRBS and gives a higher proportion of on-target reads (Figs. 1d and e). AS shows more even coverage compared than RRBS (Fig. 1f), recovers more CpGs (Figs. 1g and 1h) and is highly reproducible (Fig. 1i).

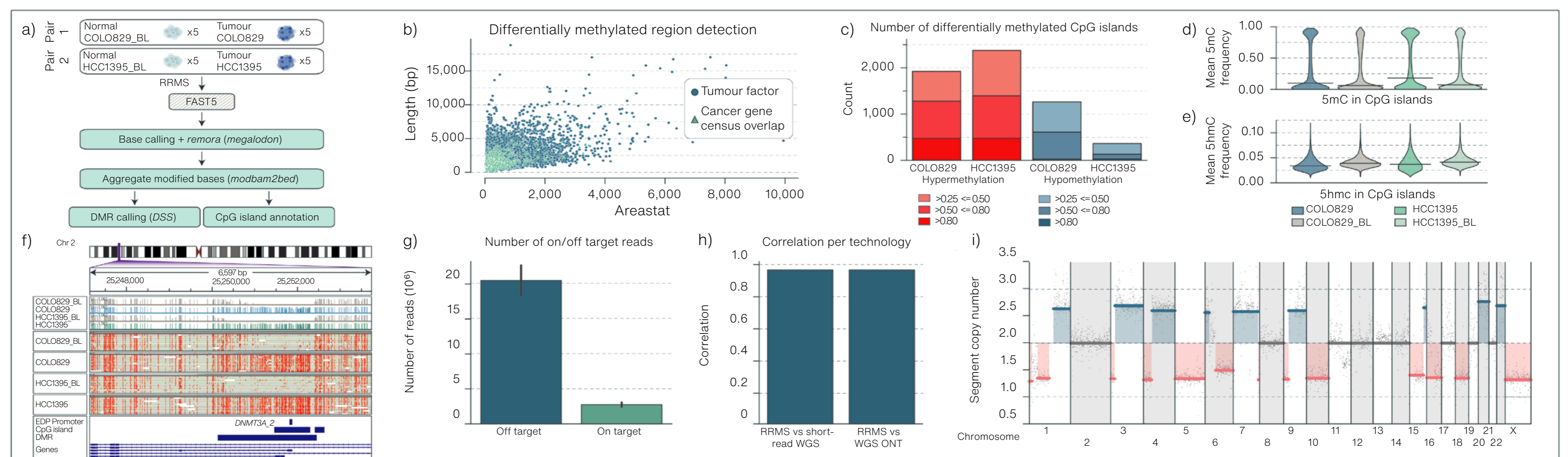


Fig. 2 Tumour/normal differences with RRMS a) workflow b) DMRs c) CpG islands d) and e) distribution of 5mC and 5hmC f) example region g) target reads h) CNV i) copy number/chromosome

Methylation patterns can be explored between samples to identify high numbers of differentially methylated features. Discarded reads can also be used to call CNV

Exploring methylation differences between tumour and normal can help to elucidate mechanisms associated with tumour formation and development. We compared methylation patterns across regions sampled by AS in a metastatic melanoma cell line and its normal pair for a male individual (COLO829/COLO829_BL) and a triple negative breast cancer cell-line pair (HCC1395/HCC1395_BL) (Fig. 2a). We were able to detect thousands of differentially methylated regions associated with the tumour sample, of which a high proportion of them overlap over >90% of their length with cancer census genes (Fig. 2b). Tumour repressor gene promoters, and overlapping CpG islands, are typically hypermethylated, which contributes to their silencing. Hypermethylation can be seen to be more abundant than hypomethylation in these regions (Fig. 2c), and this is reflected in mean methylation distribution across CpG islands (Fig. 2d). Although the role of 5hmC in cancer has been studied less than that of 5mC, nanopore technology allows us to call this modification. We are able to observe the expected reduction in the frequency of 5hmC methylation in tumour vs normal (Fig. 2e). In Fig. 2f), we show a promoter overlapping a differentially methylated region (DMR). This DMR overlaps the *de novo* methyltransferase DNMT3A and is clearly methylated in both of the tumour samples compared to their normal pairs. These methyltransferases are essential for establishing and maintaining normal levels of methylation, and so their dysregulation can contribute to cancer development. This type of analysis therefore has potential as a method of monitoring tumour progression. Finally, we show that the reads which are ejected during AS can be used to call copy-number variation across the whole genome. The AS approach therefore provides key information for tumour characterisation as well as methylation status. We see a high correlation with both whole-genome nanopore sequencing and also with short read whole genome sequencing (Figs. 2h and 2i).