Comparison of bisulfite- and methyl-capture approaches for mapping the cell-free methylome in late-stage cancer patients

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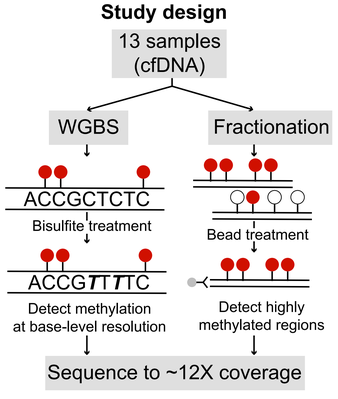
Highlights

- **Applied whole-genome bisulfite sequencing (WGBS) and fractionation approaches to assay methylation levels in cfDNA from healthy and late-stage cancer patients.**
- **WGBS, but not fractionation, detects a ~7% decrease in global methylation levels in cancer patients.**
- **WGBS finds ~3-times more CpG islands and shores which segregate cancer patients from normals.**
- **Methylation levels measured by WGBS are highly repeatable and show clear linearity of signal.**

Abstract

Due to the crucial roles methylation of DNA plays in carcinogenesis, there has been much interest in measuring these modifications in circulating cell-free DNA (cfDNA) to identify biomarkers for cancer management. We evaluated two methodologies in cfDNA collected from cancer patients and normals: (i) bisulfite conversion followed by whole-genome sequencing (WGBS); and (ii) bead-based fractionation followed by WGS of both densely hypermethylated and remaining fractions. We assessed the ability of each assay to map cancer-associated changes in methylation, both genome-wide, as well as in four specific classes of genomic regions. Globally, we observed a ~7% decrease in methylation levels via WGBS, but not using the fractionation approach. Clustering methylation levels at CpG islands and shores, but not gene bodies or promoters, led to segregation of cancer samples from normals; WGBS identified >3X regions with differences between cancer and normal samples compared to fractionation. This decreased performance by fractionation was likely due to its inability to detect changes in lowly methylated regions. Finally, we assessed the repeatability of WGBS, as well as an additional methodology - oxy-bisulfite sequencing - to specifically measure levels of 5-hydroxymethylcytosine. We observed high concordance in methylation levels between replicate samples, and found that 5-hMC levels didn't provide any utility beyond gross methylation levels as assessed by WGBS.

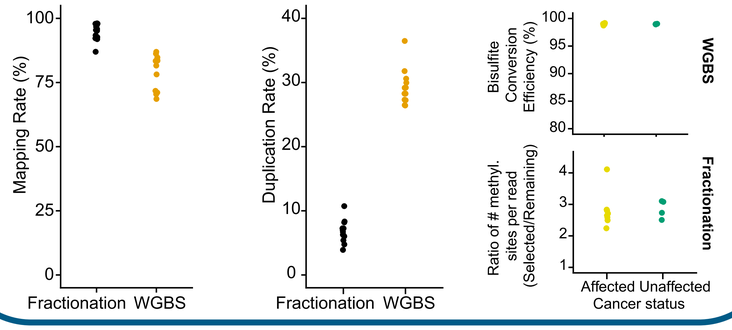
Experimental Design & QC



Participant characteristics of this study

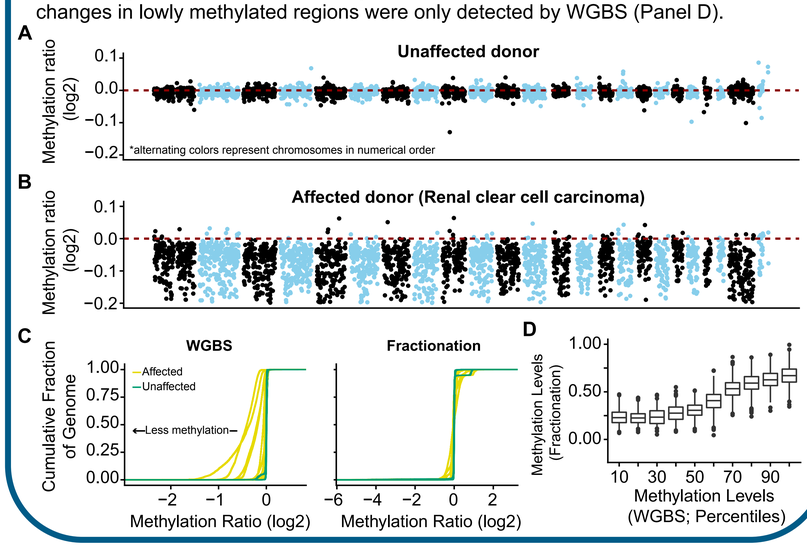
	Affected N (9)	Unaffected N (4)
Age (y)		
Median (Min, Max)	70 (49, 84)	28 (21, 59)
Sex		
Female	3	1
Male	6	3
Cancer Site		
Breast	2	
Colon	2	
Lung	2	
Rectum	2	
Renal	1	

How well does each assay perform from a technical standpoint?



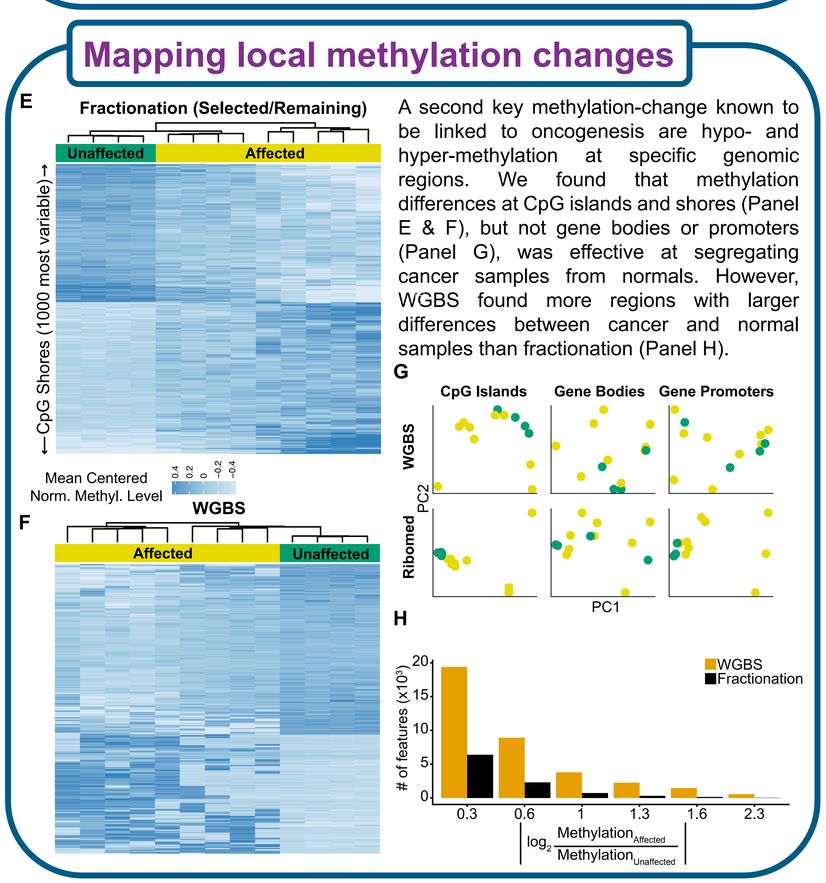
Global hypomethylation in cancer patients

Global hypomethylation is one of the hallmarks of oncogenesis. We observed a systematic decrease in methylation levels as measured by WGBS throughout the genome in cfDNA from affected patients compared to normals (Panels A & B). On average, we observed a ~7% decrease in methylation levels (Panel C; left); in contrast, almost no decrease is observed when using the fractionation data (Panel C; right). For regions with mean methylation levels in the top quartile as assessed by WGBS, we observed good concordance in methylation levels between both assays; however, most changes in lowly methylated regions were only detected by WGBS (Panel D).



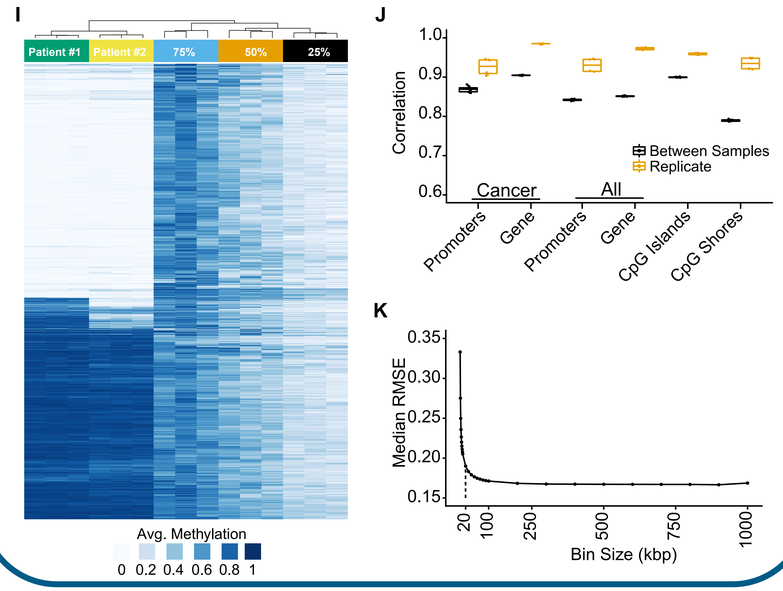
Mapping local methylation changes

A second key methylation-change known to be linked to oncogenesis are hypo- and hyper-methylation at specific genomic regions. We found that methylation differences at CpG islands and shores (Panel E & F), but not gene bodies or promoters (Panel G), was effective at segregating cancer samples from normals. However, WGBS found more regions with larger differences between cancer and normal samples than fractionation (Panel H).



Robustness & repeatability of WGBS

To assess the repeatability of WGBS, we generated libraries in triplicate from two patients with late-stage cancer (Patient #1 and #2), as well as "synthetic" samples consisting of artificially methylated DNA mixed with hypomethylated DNA in specific proportions (25%, 50% and 75%). We observed significantly higher concordance ($P<0.05$; Mann-Whitney U test) between replicates, in methylation levels across numerous genomic regions (Panels I & J). More over, we observed a clear linearity of signal, down to a resolution of 20 kbp, amongst our synthetic samples (Panel K).



Mapping 5-hMC changes in cfDNA

To assess the levels of 5-hydroxymethylcytosine (5-hMC) in cfDNA, we performed oxy-bisulfite sequencing (oxBS) in the same cohort of patients. Whereas, WGBS measures levels of both 5-hMC and 5-mC, oxBS measures only 5-mC; thus, by subtracting measurements made in oxBS from WGBS, we can quantify 5-hMC levels. While we were unable to detect any 5-hMC at the resolution of single CpG sites (likely due to insufficient read depth; Panel L), mean 5-hMC levels across multiple genomic regions of interest ranged from 1-4% (Panel M). Clustering samples based on 5-hMC levels in these regions showed modest separation between cancer and normals, however, the extent of separation was no better than methylation levels derived from WGBS (Panels N-Q).