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Cell-free DNA based methylation profiling for early cancer detection with Predicine methylation assay

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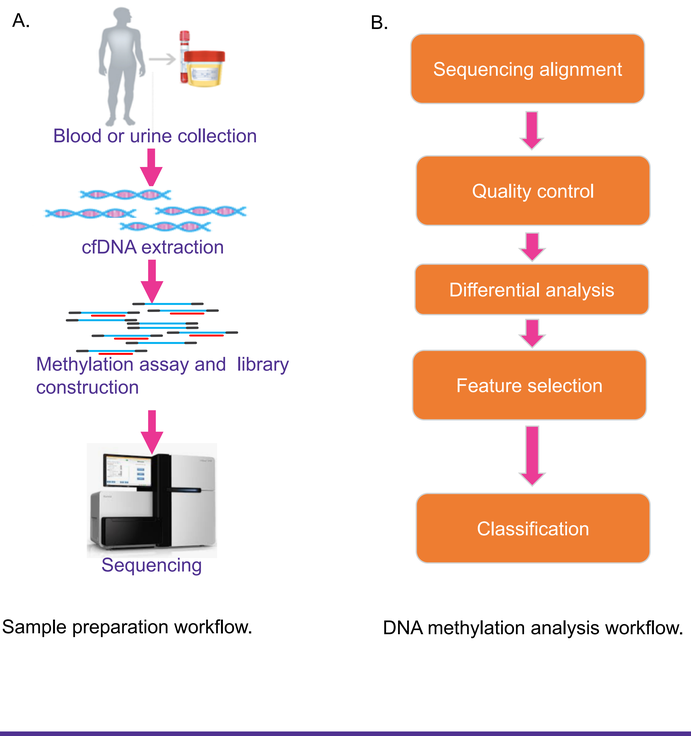
INTRODUCTION

DNA methylation is one of the earliest signatures during cancer development. DNA methylation patterns from different cancer types can provide distinct epigenetic signatures. Several methods are available for sequencing DNA methylation throughout the genome, including whole-genome bisulfite sequencing (WGBS) and antibody-dependent DNA immunoprecipitation (MeDIP). As a non-invasive approach, plasma cfDNA and urinary cfDNA (ucfDNA) have been widely used for clinical applications. More recently, coupled with cancer-specific methylation signatures, cfDNA has been adopted for early detection of cancer and identification of cancer tissue origin.

METHODS

Predicine has developed a PredicineECM (enzyme-controlled methylation) assay that can robustly detect methylation not only from genomic DNA (gDNA), but also from plasma and urinary cfDNA. Cell line gDNA with known methylation profiles, plasma cfDNA, and ucfDNA from patients and healthy donors were used in our methylation study. Methylation results were verified by comparing with public and in-house WGBS data.

Fig. 1: Workflow for PredicineECM assay.

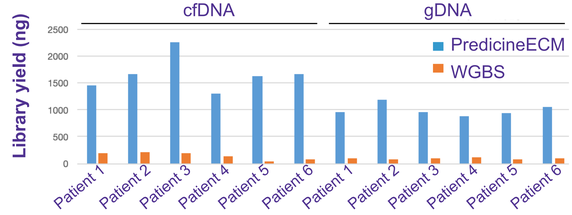


Sample preparation workflow.

DNA methylation analysis workflow.

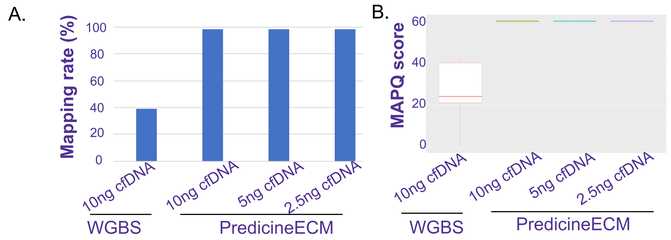
RESULTS

Fig. 2: The PredicineECM assay had much higher library yield compared to the WGBS assay with the same amount of DNA and number of PCR cycles.



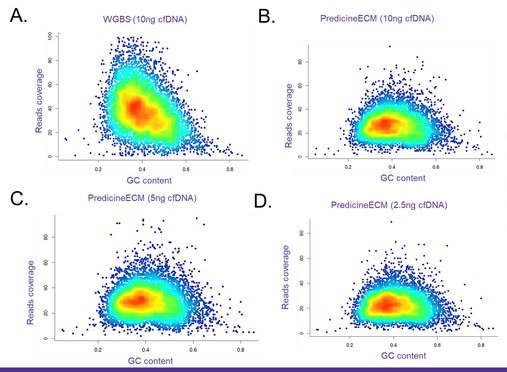
Improved library yield in the PredicineECM assay. The same amount of cfDNA or gDNA from six patients was subjected to the PrecicineECM assay and WGBS assay with the same number of PCR cycles.

Fig. 3: The PredicineECM assay had a higher mapping rate and mapping quality compared to the WGBS assay.



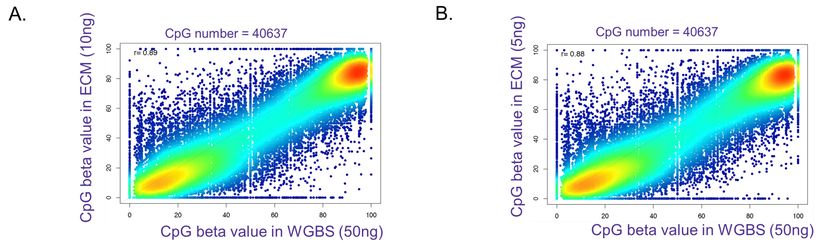
Improved mapping rate and quality with the PredicineECM assay. 2.5-10 ng cfDNA from a prostate cancer patient was subjected to the PrecicineECM assay. 10 ng of the same cfDNA was also subjected to the WGBS assay. With similar total sequencing reads, the mapping rate (a) and mapQ score (b) are significantly higher with the PredicineECM assay.

Fig. 4: The PredicineECM assay had better sequencing uniformity than the WGBS assay.



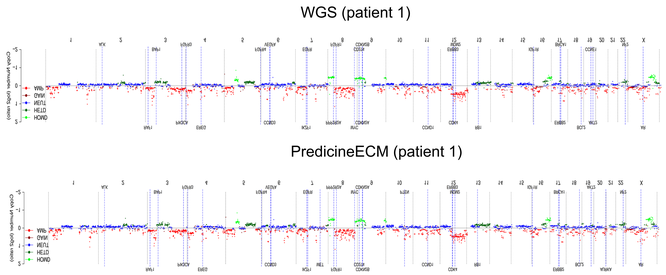
More uniform overage in the PredicineECM assay. Reads coverage across different GC content in WGBS assay with 10 ng cfDNA (a), using the PredicineECM assay with 10 ng cfDNA (b), 5 ng cfDNA (b), or 2.5 ng cfDNA from the same patient.

Fig. 5: CpG Methylation signals detected by the PredicineECM assay were highly correlated with those detected by WGBS.



High concordance of CpG beta value between the PredicineECM and WGBS assays. 10 ng (a), 5 ng (b), and 2.5 ng cfDNA (c) from a prostate cancer patient were subjected to the PrecicineECM and WGBS assays. The correlation of CpG beta values between the PredicineECM and the WGBS assays with 50ng cfDNA from the same patient was analyzed. The Pearson's r value is shown.

Fig. 6: The PredicineECM assay data can be used for Copy Number Variation (CNV) analysis.



Consistent CNV results observed between WGS and PredicineECM assays. cfDNA from a prostate cancer patient was subjected to whole-genome sequencing directly (a) and the PredicineECM assay (b) for detection of copy number variation (CNV).

CONCLUSIONS

We have developed a proprietary PredicineECM methylation assay that has dramatic improvement over WGBS in reducing DNA damage and GC bias as well as increasing NGS reads mapping rate and mapping quality score. It enables robust detection of methylation not only from gDNA, but also from low-input cfDNA. Together with the other gene profiling assays in Predicine's portfolio, PredicineECM methylation assay is potentially a powerful tool for early cancer detection and real-time monitoring of cancer progression.