# Comprehensive, simultaneous genetic and epigenetic profiling of cell-free DNA in hepatocellular carcinoma

biomodal

## Introduction

Hepatocellular carcinoma (HCC) arises from liver inflammation associated with chronic liver disease (CLD). However, the transition steps and mechanisms driving CLD to HCC are not well understood. New genetic and epigenetic research methods are revealing the complex mutational landscape of hepatocytes which may permit early detection or prevention of HCC emergence, and define the stepwise evolution of HCC from CLD[1].

Circulating tumor biomarkers present in the blood and the ability to access them repeatedly by liquid biopsy hold great promise for monitoring and understanding cancer progression. To explore the potential of using cell-free DNA (cfDNA) to identify genetic and epigenetic changes that may herald this transition to cancer, the Dawson lab at the Peter MacCallum Cancer Centre and University of Melbourne used the duet multiomics solution evoC to generate a 6-base genome from a cohort of healthy, CLD, and HCC patients across a range of disease stages. The 6-base genome generated by duet evoC delivers the most biologically relevant information from scarce cfDNA samples. It identifies A, T, C, G, 5-methylcytosine (5mC), and 5-hydroxymethylcytosine (5hmC), combining genetic sequence with methylation data at single-base resolution on the same DNA molecule and is a powerful approach to explore the relationship between genetic variants and changes in DNA methylation.

## Study Design

Samples were collected from patients with stages 0/A, B, and C/D hepatocellular carcinoma (HCC, n=120) chronic liver disease (CLD, n=70) and healthy controls (n=30) and a limited set of these were included in this initial study. For each patient, 20 ng of cell-free DNA was extracted from plasma collected in Streck tubes. Sequencing libraries were prepared using duet evoC followed by sequencing. SNP and methylation calls were analyzed using duet software v1.1.1.

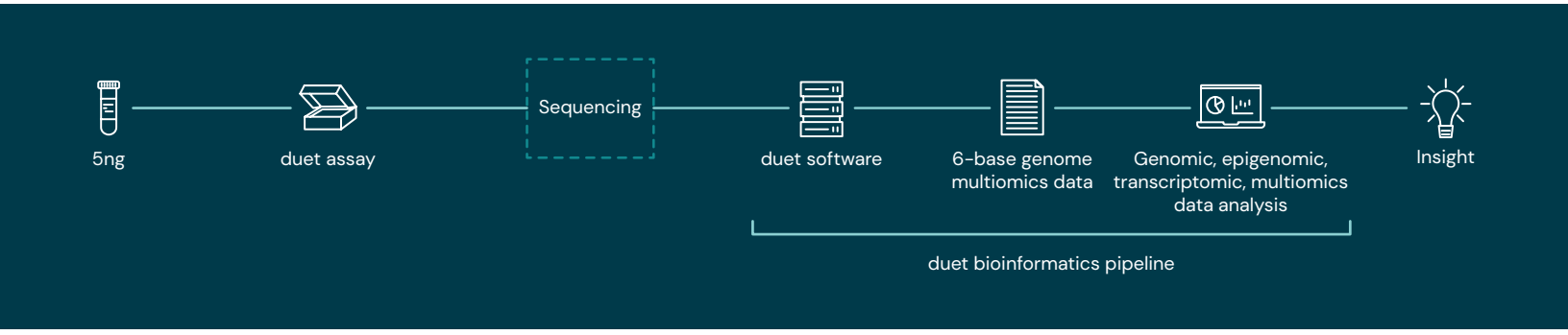


**Figure 1:** duet evoC is comprised of a pre-sequencing workflow requiring as little as 10ng gDNA or 5ng cfDNA that seamlessly integrates with NGS sequencing platforms and a post-sequencing software package to accelerate data analysis. The post-sequencing software trims, aligns, and annotates to generate analysis-ready BAM, VCF, BedMethyl, and ASM files. Additional plugins include proprietary software that facilitates analysis of multimodal data at scale.

# Results

## Sample QC analysis

Highly accurate SNP and methylation calls are important for clinical samples. Accuracy of SNP and methylation calls were assessed using Q scores at all four DNA bases across samples in batches or according to disease state (Figure 2). Results showed limited batch-to-batch variability and good reproducibility with no overt loss of performance between samples from healthy, CLD or HCC patients.

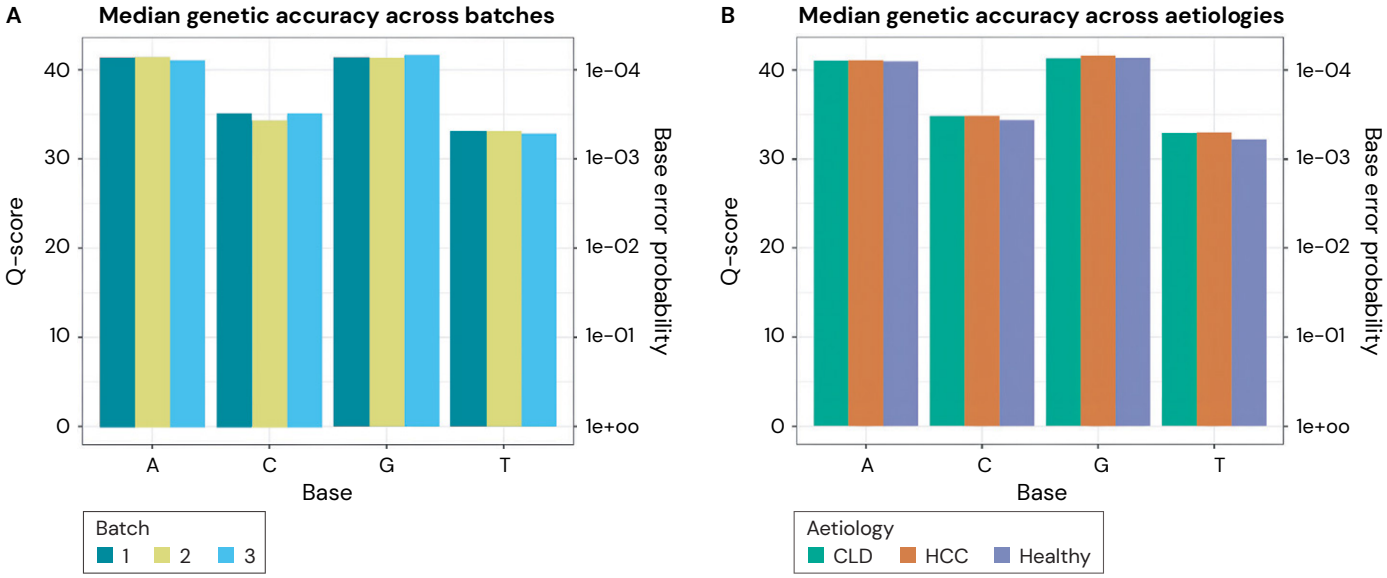


**Figure 2:** SNP and methylation Q score and error probability of all four bases. A. Median genetic accuracy across batches. B. Median genetic accuracy across aetiologies.

## Copy number analysis

The liver has well known regenerative capacity and ability to withstand repeated damage. While polyploidy, a state where the cell nucleus contains multiple complete pairs of chromosomes, is associated with genetic instability in some cells, many hepatocytes exist in polyploid states considered protective against loss of heterozygosity. However, chronic hepatocyte injury results in increased polyploidy over time[2,3]. To understand which genetic regions are involved in deviations from the diploid state and how these might influence progression from CLD to HCC, copy number analysis was performed on a subset of HCC samples at different stages, shown in Figure 3.

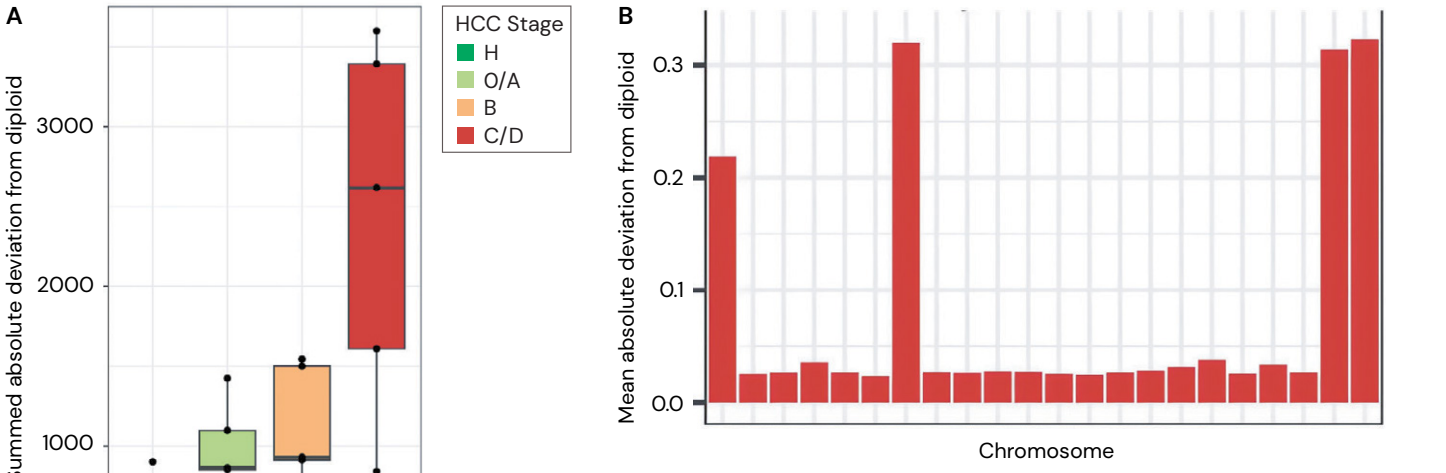


**Figure 3:** A. Values representing summed deviation from diploid are plotted on the y-axis from healthy individuals (H) and patients with stages O/A, B, and C/D HCC (n=21). B. Example of change from diploid in HCC stages C/D at all chromosome locations (x-axis) plotted against median absolute deviation from diploid (y-axis). Copy number alterations (CNAs) were visualized using QDNAseq.

## Using differentially methylated cytosines to characterize HCC vs healthy controls

To test the ability of 6-base genome data to identify differential methylation in both 5mC and 5hmC, volcano plots were generated across all potential methylation sites (Figure 4). These plots show differentially methylated cytosines (DMCs) between healthy and HCC samples. Interestingly, a large differential was observed, most notably with a reduction of 5hmC methylation in HCC versus healthy samples. A large number of DMCs were also detected for 5mC in HCC samples with sites of both increased and decreased methylation observed across the genome.

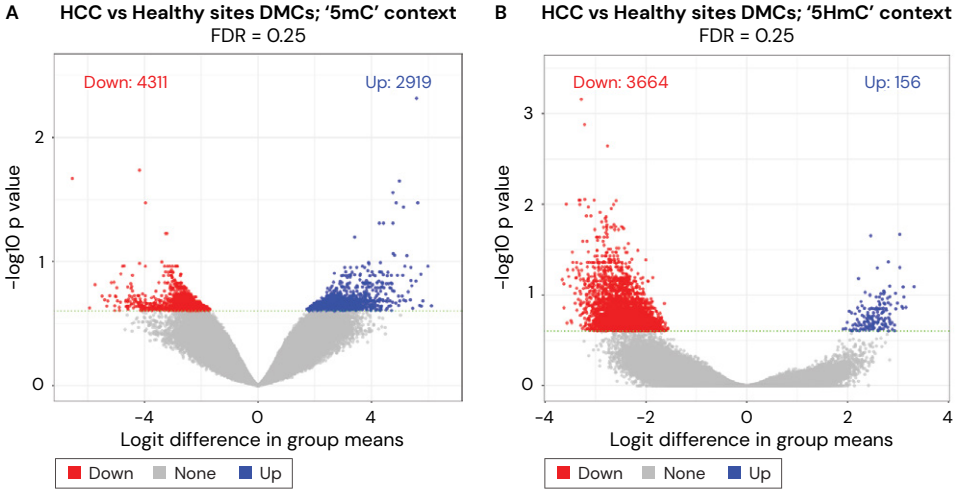


**Figure 4:** Volcano plots for all DMCs identified between healthy and HCC samples. A. 5mC DMCs plotted. B. 5hmC DMCs plotted. DMC data was analyzed using DMRcate and BSSeq packages. n=17 (HCC 11 and Healthy 6).

## Exploring epigenetic changes in promoters

The patterns of differential methylation across different genomic locations were then explored across a larger subset of samples. When focused on promoters, the majority of DMCs showed a consistent reduction in 5hmC, as with the earlier genome wide data, however, distinct patterns emerged between healthy samples, CLD and early vs late stage HCC patients (Figure 5).

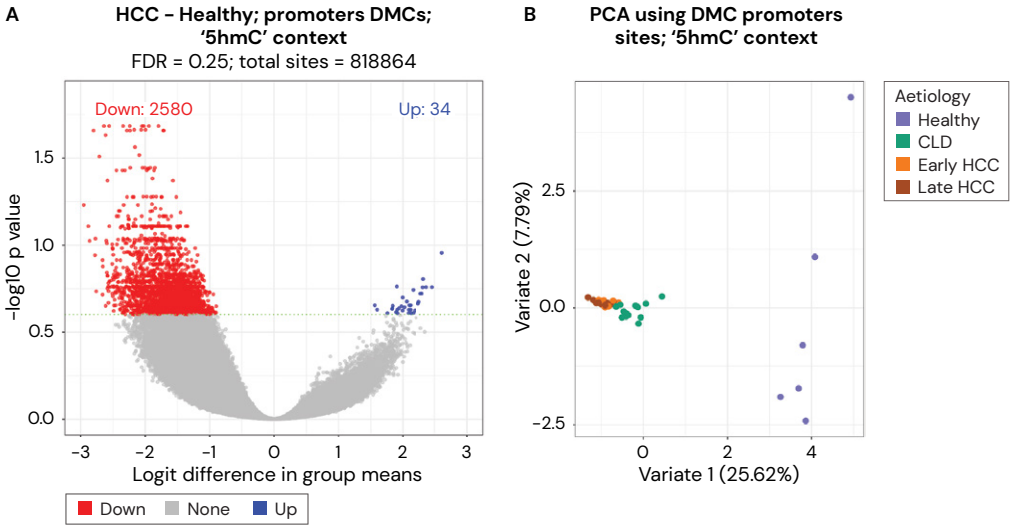


**Figure 5:** A. Volcano plot of 5hmC DMCs in promoter regions indicate a large reduction in 5hmC. B. PCA plot of DNCs identified in promoter regions for healthy, CLD, early HCC, and late HCC. Healthy vs HCC n=41 (healthy 6, HCC 21, CLD 14).

## Conclusion

In this application note, researchers demonstrate high genetic and epigenetic accuracy for analysis of clinical cell-free DNA samples in a single assay. This study suggests that profiling cfDNA with duet evoC would be very useful for analysis of liquid biopsy samples. The data shows the ability to profile multimodal features from which to build potential classifiers for HCC, including CNAs relevant to HCC biology and base-level 5mC and 5hmC information that reveal differentially methylated regions across the genome.

Future studies are underway to determine if serial monitoring of circulating tumor DNA in hepatocellular carcinoma will facilitate early diagnosis, enable real time monitoring of residual disease following treatment, and provide a comprehensive genomic profile that can assist in guiding therapeutic decisions to improve cure rates for patients.

## Ordering information

| Catalogue number | Product name | Product description |
|---|---|---|
| 6101Data | duet multiomics solution evoC: 8x reaction | Pre-sequencing workflow + post sequencing software for 8 reactions |
| Data | UDI: 8x reaction | UDIs for 8 reactions |
| Data | UDI: 96x reaction | UDIs for 96 reactions |


1. Zanotti, S *et al.* The role of chronic liver diseases in the emergence and recurrence of hepatocellular carcinoma: An omics perspective. *Front Med,* Jun 24 (2021).
2. Müller, M *et al.* Ploidy dynamics increase the risk of liver cancer initiation. *Nat Commun* 12, 1896 (2021).
3. Wang, N *et al.* The controversial role of polyploidy in hepatocellular carcinoma. *Onco Targets Ther* 14, 5335 (2021).


**Disclaimer**

The duet multiomics solution is for research use only.


**biomodal**
Chesterford Research Park
Cambridge, UK
CB10 1XL

+44 (0) 1223 800 700
biomodal.com
info@biomodal.com