Performance characterization of a novel whole genome sequencing informed MRD assay

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Poster PP.15

Introduction

Circulating tumor DNA (ctDNA) analysis for minimal residual disease (MRD) detection is increasingly demonstrating value in cancer patient care.

In some clinical settings, ctDNA levels are relatively high, and a broad range of assays are likely suitable[1,2].

However, there is growing evidence that detection down to 1 ppm (parts per million) and below will be required for many clinical applications[3].

In order to enable MRD detection in patients with lower levels of residual disease, we have developed a novel tumor informed assay and applied this to both cell line dilutions and cancer patient material.

Method

We have developed a next generation, tumor-informed targeted assay, suitable for ultra-sensitive detection of ctDNA from blood plasma and other fluids.

Upfront FFPE whole genome sequencing (WGS) of a patient's tumor is used to detect patient-specific somatic variants, then used to curate and synthesize a panel targeting up to 5,000 mutations with a proprietary variant selection algorithm.

The assay generates sequencing libraries with a novel chemistry. It can utilize a range of DNA input levels but throughout this study we added 5,000 amplifiable copies of the genome as measured by ddPCR.

The panels are then used for targeted enrichment of libraries, before sequencing. Following sequencing, reads are demultiplexed and aligned then interrogated for the known patient-specific mutations using NeoGenomics proprietary algorithms.

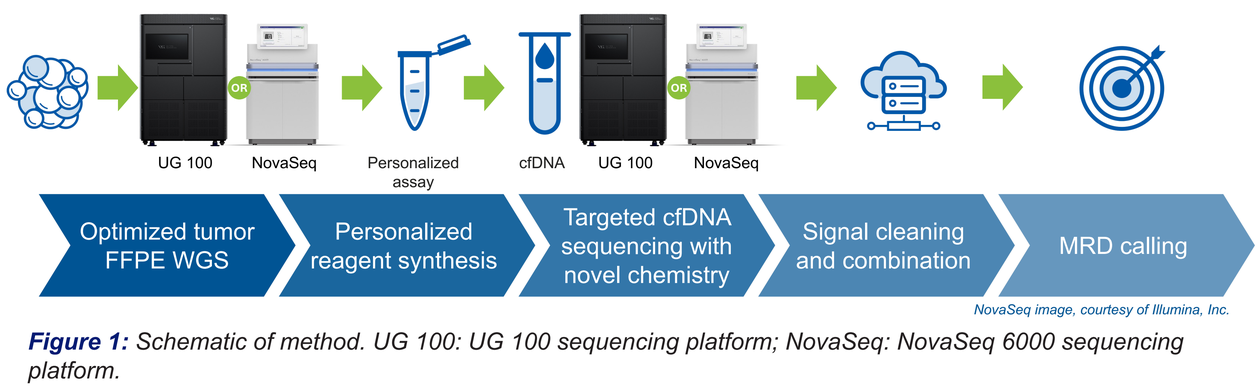


Figure 1: *Schematic of method. UG 100: UG 100 sequencing platform; NovaSeq: NovaSeq 6000 sequencing platform.*

Enzymatically digested DNA is a better surrogate for cfDNA than sonicated DNA

For performance testing and potential future validation, we wished to generate genomic material with a comparable noise profile to cfDNA. Sonicated Genome in a Bottle (GiaB) gDNA using a Covaris S220 showed higher noise, especially C>N mutations (Figure 2).

In contrast, enzymatic digestion (NEBNext UltraShear®) reduced noise levels in all classes to be comparable or lower than seen in healthy donor cfDNA (HD cfDNA).

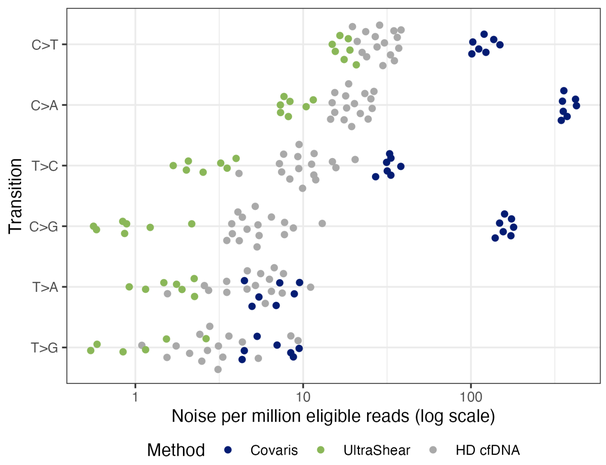


Figure 2: *Comparison of noise between Covaris sonicated gDNA, UltraShear digested gDNA and healthy donor cfDNA. Non-reference bases within 400bp of target variants were considered, excluding known poorly mapping regions and variants seen in more than 4 reads within a sample. Each point is one replicate with a 2000 variant panel, sequenced on an Illumina NovaSeq 6000.*

Selection of variants from tumor material can be performed on Illumina or Ultima platforms

Exome sequencing typically enables identifying between 20 and 200 mutations. To generate large panels of up to 5,000 variants, the assay leverages WGS of the tumor tissue. Comparison of the Illumina NovaSeq 6000 with the Ultima Genomics UG 100 shows high concordance of mutations detected as well as observed Variant Allele Frequency (VAF).

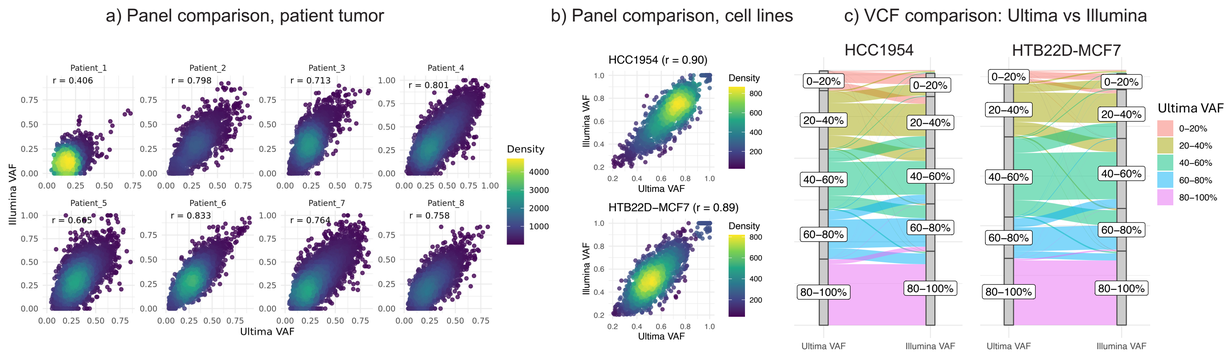


Figure 3: Comparison of VAF for WGS variants identified through Illumina and Ultima sequencing of the same samples. a) Panels of up to 5,000 variants derived from patient tumour FFPE tissue, designed on the Ultima platform and compared to their corresponding Illumina sequencing. b) Panels of ~2,000 variants derived from cell lines. c) Alluvial plot showing the comparison between all variants found in cell lines across platforms.

Cell line dilutions show high sensitivity and specificity

UltraShear digested DNA from two breast cancer cell lines, HCC1954 and MCF7, were diluted into GiaB background at concentrations between 0 and 100 ppm. These were profiled using panels of 1900 variants, alongside five HD cfDNA samples.

As shown in Figure 4, with basic calling, noise was present at ~10 ppm (~1 in 100,000). Following custom analysis with advanced filtering, positive signal was seen in all replicates at 1.2 ppm and above, with quantitative agreement with expected ppm values (Figure 4). Even at lower tested dilutions (1.2 ppm and below), most samples had two positive variants (Table 1). By comparison, many of the negative samples (healthy donor cfDNA and GiaB) had no signal, and in those that did it was always below 1 ppm.

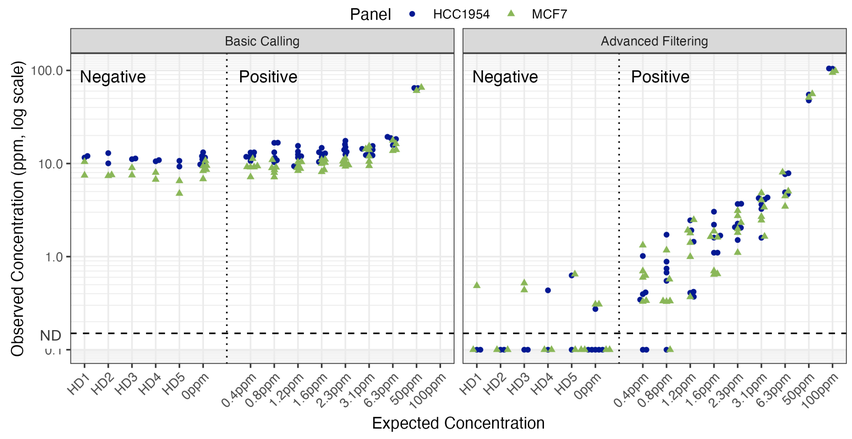


Figure 4: *Observed signal in cell line dilutions into GiaB (6 replicates except 50 and 100 ppm), as well as 5 HD cfDNA samples (2 replicates). Left side shows basic processing, right side custom NeoGenomics algorithm. Sequencing performed on an Illumina NovaSeq 6000. ND = Not Detected.*

Dilution (ppm)	Variants Detected Mean (95% CI)
HD cfDNA	0.30 (0.1, 0.5)
0	0.25 (0, 0.5)
0.4	1.50 (0.8, 2.2)
0.8	1.92 (1.1, 2.7)
1.2	3.67 (2.4, 5.0)
1.6	4.67 (3.2, 6.1)
2.3	6.58 (5.3, 7.9)
3.1	10.1 (8.2, 12.0)
6.3	17.8 (13.4, 22.1)
50	163 (148.1, 177.4)
100	276 (258.3, 293.7)

Table 1: *Number of variants detected per sample. Values are the mean with a 95% confidence interval. Values from both panels are combined.*

Variants which are called are stochastic between individual samples

At low levels of tumour fraction, we expect the detection of each individual locus to be stochastic. As shown in Figure 5, we see different variants being called in each sample, confirming that our signal is not being biased by focal copy number events.

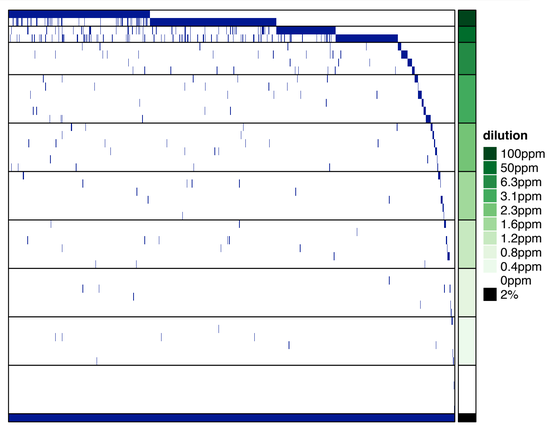


Figure 5: *Oncoplot showing individual positive variants in each MCF7 sample. Each row is one sample; each column is one of the 802 variants called in at least one of the samples. Bottom row is a 2% dilution, showing all variants are positive.*

Sequencing cfDNA on the UG 100 platform gives similar performance

HCC1954 dilution samples were converted for sequencing on the Ultima Genomics UG 100 platform. The analysis shows a very similar performance to the NovaSeq 6000, with detection below 1 ppm, good linearity and matching to expected levels and no or low signal in negative samples (Figure 4).

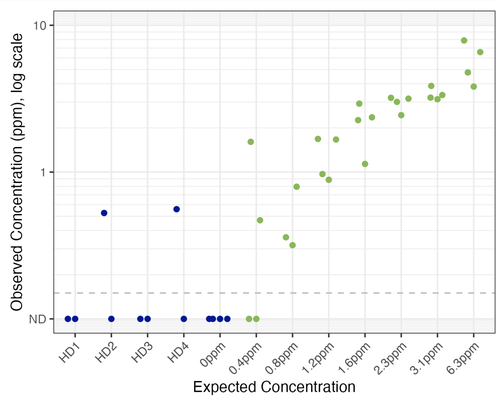
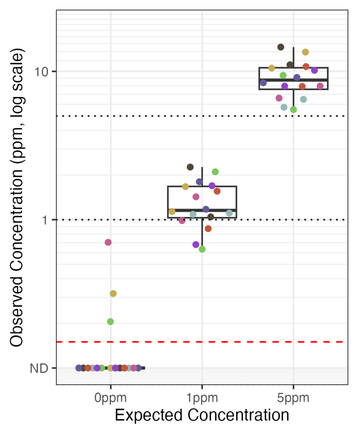


Figure 6: *Performance of a subset of samples from Figure 4 sequenced on the UG 100, showing comparable performance to Illumina sequencing. ND = Not Detected.*

Dilutions of patient cfDNA confirms ultra-sensitive detection

For 8 patients with cancer (4 HNSCC, 4 NSCLC), we designed 5,000 variant panels from UG 100 data. We then tested these in mixtures of patient cfDNA into healthy donor cfDNA at 0, 1 and 5 ppm levels, in duplicate (Figure 7). This shows the assay's ability to call MRD at levels of 1 ppm, while keeping high specificity.

Table 2 shows the number of detected variants when subsampling variants included in the panels (*in silico*).



Panel Size	0ppm	1ppm	5ppm
100	0.006	0.12	0.79
500	0.02	0.58	4.00
1000	0.05	1.18	7.91
1500	0.07	1.74	11.9
2000	0.09	2.31	16.0
3000	0.16	3.43	23.6
5000	0.25	5.00	34.4

Table 2: *Mean number of variants detected as the panel size is decreased. For panel sizes less than 5,000, 100 bootstraps were generated for each sample.*

Figure 7: *Observed ppm signal in dilutions of patient cfDNA mixed into HD cfDNA. Eight patient panels of up to 5,000 variants, all samples in duplicate. Color represents the panel used in each sample. ND = Not Detected.*

Conclusion

We are developing a WGS-based tumor-informed assay for MRD detection, which is able to consistently detect cancer signal at levels in the order of 1 ppm and below, through novel chemistry, proprietary algorithmic selection of variants and bioinformatic processing. We have shown the assay is compatible with both Illumina and Ultima sequencing, both for variant selection in tumor and MRD assessment in cfDNA. We continue to refine the molecular and algorithmic processes to reduce the false positive calls and boost signal.

References [1] Ruiz-Torres DA, et al. NPJ Precis Oncol. 2025 Aug 22;9(1), [2] Fuentes-Antrás J, et al. NPJ Breast Cancer. 2025 Jul 19;11(1) [3] Schuurbiers MMF, et al. PLoS Med. 2025 Apr 15;22(4)

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