Single stranded ligation enhances the performance of the duet evoC 6-base assay, enhancing value in low DNA input applications including liquid biopsy

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1. Introduction

Circulating cell-free DNA (cfDNA) contains rich genetic, epigenetic, and structural information for cancer detection, but its highly fragmented nature, low abundance, and high background make reliable analysis challenging. Conventional double-stranded DNA (dsDNA) workflows rely on end repair and fragment reconstruction, which can lose damaged or ultra-short molecules and introduce artefacts, limiting sensitivity and increasing the limit of detection (LoD), particularly at ultra-low ctDNA fractions.

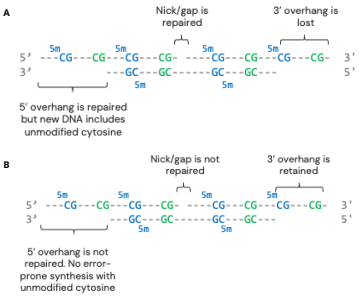

Fig. 1 Molecular impact of dsDNA versus ssDNA library preparation workflows. (A) Double-stranded workflows are error-prone and repair fragments with unmodified cytosines. **(B)** A single-stranded ligation duet workflow. Original cfDNA features are retained, with no new DNA synthesised.

biomodal's single-stranded DNA (ssDNA) workflow is optimised for cfDNA, capturing damaged and ultra-short fragments by ligating adapters directly to single-stranded molecules without end repair. High material recovery combined with precision 6-base sequencing enables accurate and specific variant and methylation calling, improving reproducibility and lowering LoD. duet evoC cfDNA further integrates phased multiomic readouts from the same molecules—including genetic variants, independent 5mC and 5hmC, and native fragmentomic features—reducing false negatives and improving confident detection of tumour-derived signals.

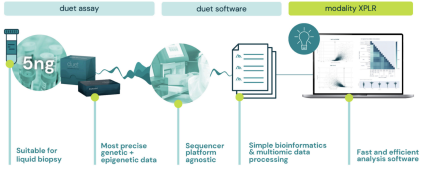

Fig. 2 duet ssDNA workflow. duet ssDNA workflow provide complete end-to-end solutions for liquid biopsy applications. The workflow includes: the duet assay, processing software and the modality XPLR software toolkit, designed for accessible, scaleable analysis of 5 and 6-base data.

2. The analytical performance of the ssDNA duet workflow enables ultra-low limit of detection (LoD)

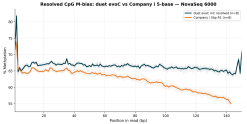

Fig. 3 M-bias plot comparing % methylation for ssDNA and dsDNA ligation approaches. A methylation bias plot showing the methylation (5mC) proportion across each position in the read. Data uses 8 libraries made with 30ng (duet cfDNA evoC) or 20ng (competitor i) Seraseq® lymphoma WT.

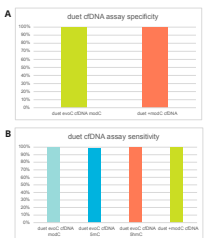

Fig. 5 duet ssDNA workflow methylation specificity and sensitivity. (A) Assay specificity was calculated as the proportion of CpGs in reads aligned to the unmethylated lambda control. **(B)** Assay sensitivity was calculated as the fraction of total versus expected mC calls in the methylated pUC genome; the sensitivity of hmC is calculated as the fraction of total versus expected hmC calls on a short oligo controls. The controls were spiked into a background of a range of cfDNA samples (real cfDNA, artificial cfDNA, Seracare cfDNA controls).

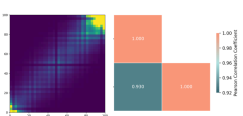

Fig. 4 Technical reproducibility. Person's correlation plot showing genome-wide pairwise correlation of per-CpG modC fractions (modC / total C, min. coverage ≥10×) across technical replicates.

A

	LoD95
Hypermethylation	6.65 ppm
Hypomethylation	12.34 ppm

B

Assay	LoD95	LoB
6-base	0.540%	0
5-base	0.497%	0

Fig. 6 Limit of detection (LoD) assessment for duet ssDNA assay.
(A) 5mC LoD. Analytical limits of detection (LoD) for methylation were evaluated using Seraseq® methylated and unmethylated ctDNA reference standards. Hypermethylated fragments were defined as ≥80% modified CpGs and hypomethylated fragments as ≤20% modified CpGs. LoD95 values were estimated using logit regression at 98% specificity, showing close concordance between experimental and modelled methylation LoD for both hyper- and hypomethylation. **(B) Genetic LoD.** LoD was evaluated using 30 ng input of Seraseq ctDNA Reference Material v4 with variant allele frequencies (VAFs) of 0%, 0.1%, 0.5% and 5%. Samples were analysed using the Genetic LoD Panel (10.4 kbp; 49/51% GC/AC content; IDT). Published tumour-naïve and MRD experiments indicates that clinically useful ctDNA tests operate in the ppm regime: MRD tests require a genetic LoD of ~10 ppm or less, and MCED methylation assays target ~44 ppm (analytical) with ~5 ppm LoB (2) with clinical cut-offs around ~100 ppm in independent methylation approaches (3). In our ssDNA duet assay, the observed LoD performance aligns with — and in methylation sensitivity can exceed — these market-relevant ppm thresholds.

3. duet with single stranded ligation has market leading methylation and genetic accuracy including detection of C>T variants

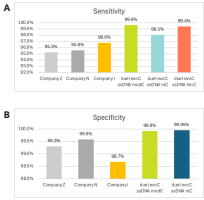

Fig. 7 Methylation sensitivity (A) and specificity (B) compared other epigenetic sequencing technologies.

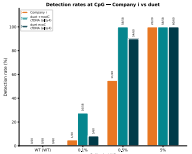

Fig. 8. Comparison of detection of C>T/G>A variations between duet ssDNA workflows (evoC /+modC) with another market player (company I). Detections rates show number of variants detected for each VAF level for CpG C>T / G>A variants.

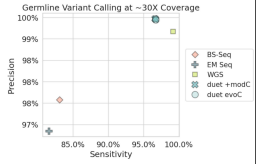

Fig. 9. Germline variant SNV calling: precision vs sensitivity. Comparison of SNV calling agains other technologies present on the market revealed that duet's ssDNA workflow matches WGS data and surpass other solutions.

4. All your cfDNA insights in one seamless workflow

In this poster, we demonstrate that a single-stranded cfDNA workflow delivers superior analytical performance for genomic and methylation (5mC/5hmC) analysis compared with existing approaches. Importantly, the same libraries preserve native fragment information, enabling fragmentomics analysis without additional assays.

Broader fragmentomic features and classifications are presented in "**6-base multi-modal data is a powerful liquid biopsy platform for cancer detection, enabling the discovery of epigenetic, genetic and fragmentomics biomarkers in a single workflow**" poster, highlighting the full potential of fragment-based biomarkers within an integrated single-assay framework.

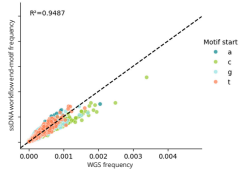

Fig. 10 cfDNA end motif frequencies are strongly correlated (~95%) between ssDNA duet and whole-genome sequencing (WGS) from matched samples.

5. Conclusions

Sensitive cfDNA analysis requires exceptional analytical performance to reliably detect tumour-derived signals at very low allele fractions. The ssDNA duet workflow achieves ultra-low limits of detection by combining high material recovery with outstanding sensitivity and specificity across genomic and epigenetic features.

- Ultra-low limit of detection (LoD) enabled by high material recovery from low-input cfDNA
- Outstanding analytical sensitivity and specificity, supporting confident detection at ultra-low tumour fractions
- Robust detection of genetic variants, 5mC and 5hmC within a single ssDNA assay
- Reduced false positives and false negatives, improving reliability at the limit of detection
- Consistent performance across genomic and epigenetic signals, supporting composite biomarker analysis

6. References

1. Füllgrabe J. et al. Simultaneous sequencing of genetic and epigenetic bases in DNA. Nat Biotechnol. 2023 Oct;41(10):1457-1464.
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3. Melton CA, Freese P, Zhou Y, Shenoy A, Bagaria S, Chang C, Kuo CC, Scott E, Srinivasan S, Cann G, Roychowdhury-Saha M, Chang PY, Singh AH. A Novel Tissue-Free Method to Estimate Tumor-Derived Cell-Free DNA Quantity Using Tumor Methylation Patterns. Cancers (Basel). 2023 Dec 23;16(1):82. doi: 10.3390/cancers16010082.

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