duet 6-base mosaic

From every fragment, the full picture

Maximize insight from every precious sample by using single-stranded ligation to read genetics, 5mC, 5hmC and fragmentomics from the same molecule. Ideal for cfDNA and liquid biopsy

Introduction

Genetics, epigenetics and fragmentomics each provide valuable insight into disease biology. Traditionally, researchers have been forced to choose one or perform multiple workflows that consume additional sample and generate disconnected datasets.

duet 6-base mosaic provides each of these data types from a single low-input workflow eliminating the need for compromise. A single-stranded ligation workflow captures short and damaged cfDNA fragments that carry important disease signal along with intact double-stranded molecules, while duet's hairpin architecture preserves confident variant detection and independently resolves 5mC and 5hmC from the same molecules.

The result is integrated genetic, complete epigenetic and fragmentomic insight from a single workflow. It is the ideal choice for cfDNA and liquid biopsy, and a powerful option for wherever sample is the limiting factor.

Key features and benefits:

- **Read genetics, 5mC, 5hmC and fragmentomics** independently from the same input molecule
- **Capture more of the molecules that matter** – single stranded workflow captures smaller and denatured DNA fragments in addition to double-stranded molecules
- **Resolve 5mC and 5hmC with high sensitivity and specificity**, reducing noise and false positive/negative signals
- **Detect genetic variants with high confidence and low LOD** – preserve the integrity of the input molecules to capture all classes of variation, including C>T mutations
- **Access native fragmentomics** – fragment ends are preserved enabling end motif, fragment length and nucleosome position analyses
- **Take advantage of built-in error correction** – hairpin design enables confident identification of PCR and sequencing errors
- **Leverage positive and negative methylation controls** built into every kit to calibrate assay performance across samples
- **Scale your liquid biopsy research** – automation friendly kit design and workflow
- **Analyze your data your way.** Run duet software on a workstation, HPC, or cloud environment using community-standard outputs and modality XPLR for biological interpretation.

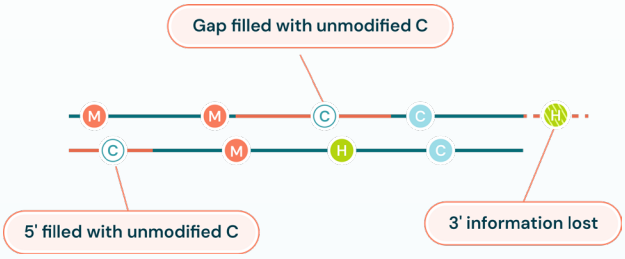
Single low-input workflow

duet mosaic's single-stranded DNA workflow is optimized for cfDNA, capturing damaged, ultra-short and double stranded fragments, by ligating adapters directly to molecules without end repair. High material recovery combined with precision 6-base sequencing enables accurate variant and methylation calling, improving reproducibility and lowering LoD.

duet 6-base mosaic improves the phased multiomic readout from the same molecules, including genetic variants, independent 5mC and 5hmC, and native fragmentomic features, reducing false negatives and improving confident detection of tumor-derived signals.

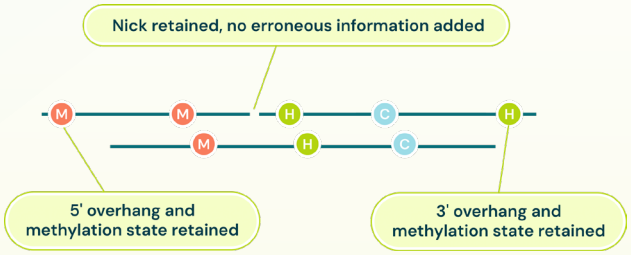
Traditional end–repair and A-tailing:

- ✗ Fills or removes overhangs
- ✗ Removes methylation signal
- ✗ Poorly captures short fragments
- ✗ Cannot capture ssDNA



Single stranded ligation:

- ✓ Retains overhangs
- ✓ Retains methylation signal
- ✓ Captures short fragments
- ✓ Captures ssDNA



duet mosaic preserves DNA fragments instead of rewriting them

Figure 1: duet mosaic's single-stranded ligation captures and preserves original cfDNA features, increasing signal-to-noise and giving you a better chance of finding the biomarkers that matter

Capture more of the informative molecules

Single-stranded ligation recovers both double- and single-stranded fragments, including the short, damaged molecules that have been proven to be enriched for circulating tumor DNA[1] that conventional double-stranded workflows leave behind.

→ **Why it matters:** Shorter cfDNA populations are enriched for ctDNA[1], by capturing more of them you get a clearer picture of disease biology and billions more chances to find disease-specific biomarkers.

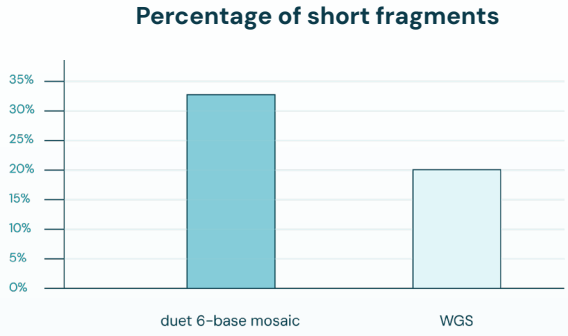
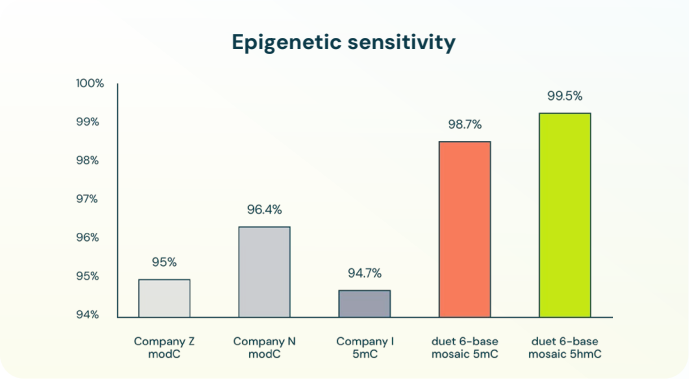


Figure 2: Quantification of short cfDNA fragments (below ~145bp) in matched clinical cfDNA samples processed using duet 6-base mosaic and a conventional end-repair and A-tailed approach shows billions more short molecules are captured with duet 6-base mosaic.

Resolve 5mC and 5hmC with high sensitivity and specificity

5mC is a mark of repression whereas 5hmC is a mark of activation, they have fundamentally different biological roles. Most methylation technologies conflate these two into a single readout or read only one, obscuring biology, hiding biomarkers and providing a compromised or partial view of the true epigenetic landscape in your sample.

With duet 6-base mosaic, read 5mC and 5hmC as distinct signals, gaining the critical insights of activated regions vs. repressed regions with market-leading sensitivity and specificity.



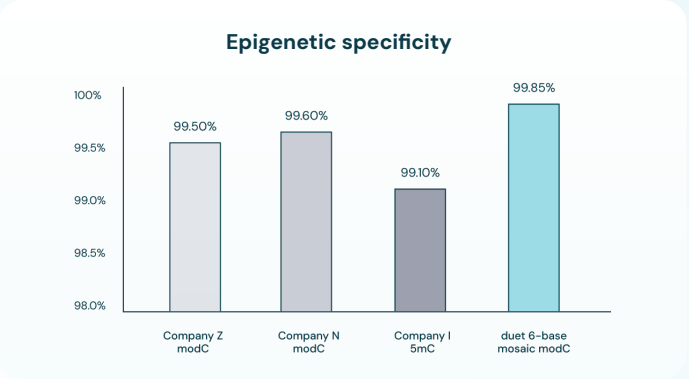


Figure 3: Methylation sensitivity and specificity* of duet 6-base mosaic evaluated using the included controls and compared to published data for other epigenetic sequencing technologies. Not only is duet 6-base mosaic the only technology to distinguish activating 5hmC from repressive 5mC, it does so with the highest sensitivity and specificity.

*Here specificity is included for modC as 6-base specificity is a three state problem whereas for other epigenetic sequencing approaches it is a two state problem, hence we have simplified the metric for a more interpretable comparison.

Why it matters: High sensitivity and specificity mean 6-fold lower false-positive methylation calls than company I and better recovery of the real methylation signal in your cfDNA sample. In a biomarker discovery setting this means 4% more methylation biomarkers can be found and helps researchers focus on real biological signal rather than analytical noise.

Detect genetic variants at low allele frequencies

Sensitive detection of individual low-frequency variants, including the C>T mutations which are most prevalent in cancer[2] (e.g. NRAS G12D[3]), while maintaining high-quality epigenetic measurements from the same molecules.

Why it matters: A typical tumour contains at least 300 C>T variants[2] which could be present in a patient's cfDNA sample at low VAF. Have confidence you can identify those low VAF variants alongside epigenetic changes, maximizing your chance of finding the biomarkers that matter.

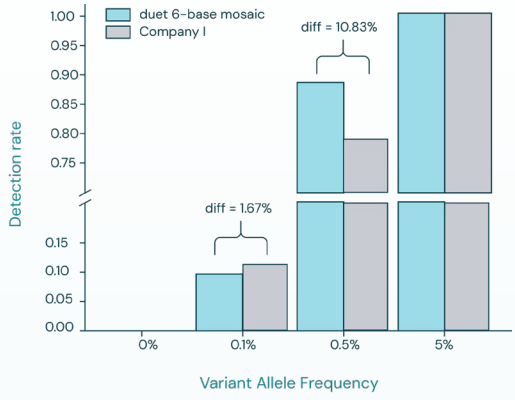


Figure 4: duet 6-base mosaic has superior C>T and G>A SNV calling performance in contrived samples of known VAF between 0% and 5%

Native fragmentomics

Fragmentomic features are only as good as the fidelity with which fragment ends are captured. Because duet 6-base mosaic's single-stranded ligation preserves native fragment ends, rather than repairing and rewriting them, as conventional double-stranded workflows do, it faithfully recovers the fragment-length distribution, end-motif frequencies, and nucleosome-positioning signals used in fragmentomic analysis. Fidelity is measured as concordance with whole-genome sequencing; end-motif frequencies correlate closely with WGS from matched samples.

With high fidelity 6-base fragmentomic data you can make full use of conventional fragmentomic biomarkers and extend them into 6-base space. Using 5mC and 5hmC aware end-motifs increases the range of biomarkers available from 256 to 4,096, enabling higher granularity and improving classifier performance[4,5].

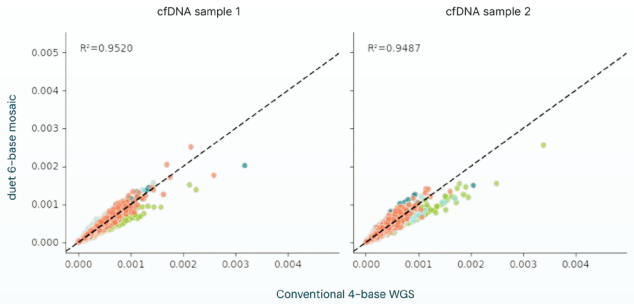
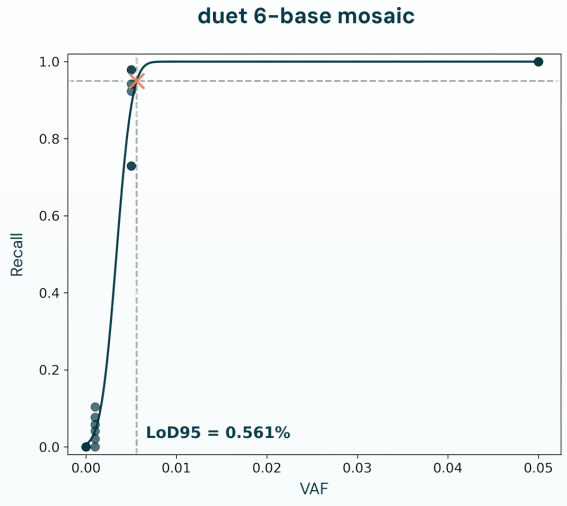


Figure 5: High correlation between the frequency of calling A (dark teal), T (coral), G (light teal) or C (green) at the 5' fragment end between conventional 4-base and duet 6-base mosaic WGS in real cfDNA samples.

Why it matters: Fragmentomic biomarkers such as fragment-size ratios, end-motif diversity, and nucleosome footprints depend on faithfully preserved fragment ends. Distorted ends from end-repair-based workflows can obscure exactly the signals these methods rely on.

Ultra-low limits of detection

Capturing more of the right molecules and high genetic and epigenetic accuracy combine to yield a lower limit of detection (LoD) than lower accuracy or single-analyte methods. duet 6-base mosaic's methylation LoDs are 4-8 fold lower than the current state of the art[6] and 10 fold lower than typical clinical sample LoD[7], with a genetic LoD that can go toe to toe with liquid biopsy market leaders[8].



	LoD95	State of the art
Hypermethylation	6.65 ppm	44ppm[6]
Hypomethylation	12.34 ppm	
Genetics	0.561%	0.2-0.8%[8]

Figure 6 and Table 1: Methylation (5mC) and single variant genetic LoD95 derived from contrived spike in samples of known VAF or methylated/unmethylated concentrations and compared to the current state of the art.

Why it matters: When signals are scarce, a lower LoD drives greater ability to accurately detect them, particularly important in the most challenging liquid biopsy early detection (ECD) and monitoring (MRD) applications.

Complete genetics and epigenetics on the same DNA fragment

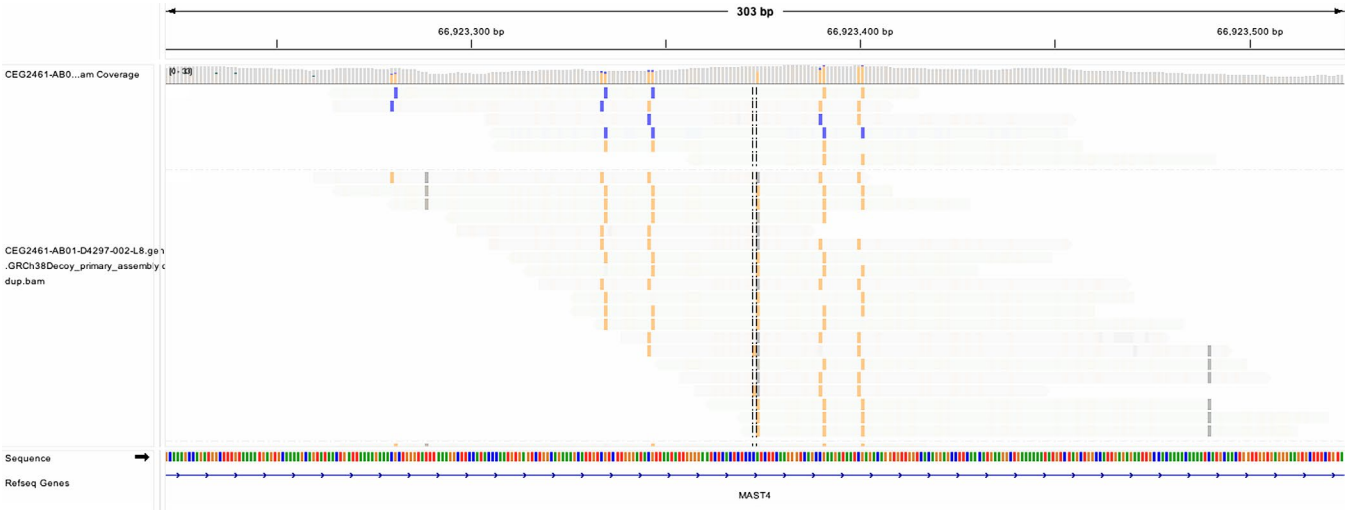


Figure 7: IGV view of a region of the genome where reads are centred on a variant (grey). The upper reads have an A variant and have CpGs with a mix of 5mC (blue) and 5hmC (yellow). The lower reads have a C variant and have CpGs that are hydroxymethylated only. This observation can be used to filter out noise. The variant and the methylation status of the fragment co-occur: C only occurs with hydroxymethylated CpGs, whereas the other variant occurs with mixed 5mC/5hmC. Pairing the variant signal with its expected methylation pattern increases specificity, which allows you to call ctDNA from fewer molecules, recovering sensitivity and lowering LoD.

Why it matters: Combining multiple signals on a single molecule enables more confident identification of ctDNA and can lower LoD.

More detection power through multiomic integration

When independent signals are assembled, they detect what any single piece misses. In a CRC cfDNA study, a duet multiomic classifier integrating genetics, methylation, and fragmentomics reclassified 6% of the samples that a methylation-only model missed, including two Stage I patients and four controls[9].

Performance gain over modC baseline	6%
Healthy samples correctly reclassified	6%
CRC samples correctly reclassified	7%

Table 2: The impact of improvements to classifier performance when moving from a modC based classifier to a full multiomic 6-base classifier

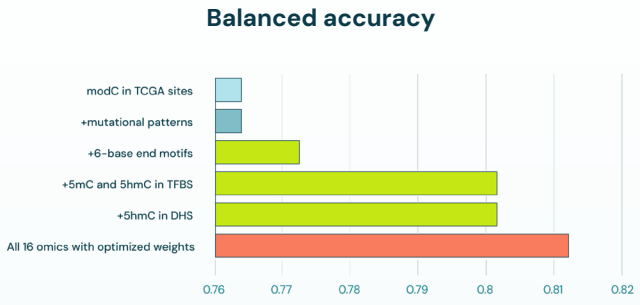


Figure 8: Multiomic integration gave stepwise improvements to balanced accuracy (the average of sensitivity and specificity) when distinguishing cancer cfDNA samples from healthy controls. The addition of 6-base biomarkers had the largest impact on performance.

Why it matters: Better ctDNA detection is needed for challenging liquid biopsy applications. Get more opportunities to find the biomarkers you need, boosting ctDNA detection and test performance and improving your ability to distinguish healthy from disease.

Software included, analysis on your terms

Every duet 6-base mosaic kit includes the duet software pipeline and modality XPLR, taking you from raw reads to resolved genetics, methylation, and fragmentomics without assembling your own pipeline.

Run it on your HPC or the cloud, and explore multiomic results on your laptop with no dedicated bioinformatician required. Analysis tools come standard, and your data stays yours.

duet assay

Suitable for cfDNA and liquid biopsy

High accuracy with low DNA requirements unlocks a powerful tool for early disease detection.

Most precise genetic + epigenetic data

Proprietary enzymatic workflow that detects 5mC and 5hmC with a sensitivity and specificity of >98%.

duet software

Sequencer platform agnostic

Compatible with most short-read instruments, no additional capital equipment required.

Simple bioinformatics & multiomic data analysis

Easily process raw sequencing data to generate the complete 6-base genome with high accuracy. Produces FASTQ, BAM, VCF, and methylation Zarr files for downstream analysis.

Fast and efficient analysis software

Optimized for scalable data exploration, differential analysis and visualisation of 6-base methylation data.

Why it matters: Get to biological insight faster, on your own infrastructure, with no custom pipeline to build and no data lock-in.

Summary

Every cfDNA fragment contains valuable biomarkers and biological information. duet 6-base mosaic brings genetics, complete epigenetics and fragmentomics together in a single workflow, helping researchers capture more of the molecules that matter and extract more insight from every DNA fragment.

From biomarker discovery to liquid biopsy assay development, duet 6-base mosaic provides the foundation for comprehensive multiomic analysis from limited input material.

Specification	Details
Sample type	Cell-free DNA (cfDNA)
Recommended input	5-30 ng cfDNA
Workflow	Single-stranded, 7 step cfDNA-optimized workflow, automation-ready. Whole genome or targeted*
Sequencing compatibility	Standard short-read sequencing platforms
Analysis	Specification

***Contact us for guidance on targeted experiments**

Ordering Information

Catalog number	Product Name	Product Description
6301	duet 6-base mosaic 8x reaction	duet 6-base mosaic assay, duet software, modality XLPR for pre and post-sequencing workflows for 8 reactions
6302	duet 6-base mosaic 24x reaction	duet 6-base mosaic assay, duet software, modality XLPR for pre and post-sequencing workflows for 24 reactions
6303	duet 6-base mosaic 96x reaction	duet 6-base mosaic assay, duet software, modality XLPR for pre and post-sequencing workflows for 24 reactions
4103	UDI 8x reactions	Unique dual indices for 8 reactions
4102	UDI 24x reactions	Unique dual indices for 24 reactions
4104	UDI 96x reactions	Unique dual indices for 96 reactions
4001	Magnetic beads mosaic 8 reaction	Magnetic beads for 8 reactions
4002	Magnetic beads mosaic 24 reaction	Magnetic beads for 24 reactions
4003	Magnetic beads mosaic 96 reaction	Magnetic beads for 96 reactions

1. *Mouliere, F. et al. Enhanced detection of circulating tumor DNA by fragment size analysis. Sci. Transl. Med. 10, eaat4921 (2018).*
2. *Alexandrov, L. B. et al. The repertoire of mutational signatures in human cancer. Nature 578, 94–101 (2020).*
3. *VCV000039648.32 - ClinVar - NCBI. https://www.ncbi.nlm.nih.gov/clinvar/variation/39648/.*
4. *Zhou, Q. et al. Epigenetic analysis of cell-free DNA by fragmentomic profiling. Proc. Natl. Acad. Sci. U. S. A. 119, e2209852119 (2022).*
5. *Abstract 122: Using the 6-base genome for full multiomic analysis of cfDNA through combined methylation and fragmentomic analysis to enhance classification of clinical cancer cfDNA samples. | Cancer Research | American Association for Cancer Research. https://aacrjournals.org/cancerres/article/86/7_Supplement/122/775973.*
6. *Melton, C. A. et al. A Novel Tissue-Free Method to Estimate Tumor-Derived Cell-Free DNA Quantity Using Tumor Methylation Patterns. Cancers 16, 82 (2023).*
7. *Hong, T. H. et al. Clinical Utility of Tumor-Naïve Presurgical Circulating Tumor DNA Detection in Early-Stage NSCLC. J. Thorac. Oncol. 19, 1512–1524 (2024).*
8. *P200010S008B.pdf. https://www.accessdata.fda.gov/cdrh_docs/pdf20/P200010S008B.pdf.*
9. *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 | biomodal. https://biomodal.com/poster/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/ (2026).*