Ultra-sensitive Synchronous Detection of Methylation and Mutation | LeXDual Comprehensive Solution

Background

DNA methylation, as a chemical modification that does not alter the DNA sequence but can influence gene activity, plays a critical role in various biological processes, including gene expression regulation, embryonic development, cell proliferation and differentiation, and maintaining genomic stability. Abnormal DNA methylation is present throughout the entire process of cancer initiation and progression. Compared to traditional tumor markers, DNA methylation markers offer advantages such as earlier detection, less invasiveness, and greater precision. These markers are well-suited for early cancer diagnosis, risk assessment, minimal residual disease (MRD) monitoring, and guiding therapy.

Currently, DNA methylation detection primarily relies on bisulfite (BS) conversion and enzymatic conversion techniques. BS conversion is fast and efficient, but the drastic changes in temperature and pH during the process can lead to DNA degradation and fragmentation, making it more suitable for samples with higher initial input amount. Enzymatic conversion is relatively mild but involves cumbersome and time-consuming procedures, with less stable conversion efficiency.

DNA methylation detection based on NGS offers high throughput, making it suitable for the detection of single cancer type, multiple cancer types, or pan-cancer types. However, limited by the low initial amount of cfDNA samples and the need for simultaneous mutation detection, the operation of multi-omics analysis is complex and the high costs.

To achieve the synchronous detection of methylation and mutation signals from a single, limited sample, LexigenBio has launched the **ultra-sensitive solution for synchronous detection of methylation and mutation: LeXDual Comprehensive Solution**. This solution only requires one initial sample, one hybridization reaction, and one set of capture probes to achieve an ultra-highly sensitive panoramic detection of methylation and mutation.

Introduction

LeXDual Comprehensive Solution integrates methylation sensitive restriction endonuclease (MSRE) technology with the exclusive patented LeXso Hybrid System, aiming to achieve ultra-high sensitivity in the synchronous det- ection of DNA methylation and mutation from a single sample. This solution utilizes MSRE technology, which specifically cleaves unmethylated sites; when a specific base within the recognition site is methylated, the cleavage is blocked. As a result, this approach can simultaneously generate both enzyme-digested and undigested methylated DNA libraries from the same sample. These libraries can then be used in the LeXso Hybrid System for the synchronous detection of DNA methylation and mutation within a single day. **LeXDual Comprehensive Solution** not only includes all the necessary reagents for the entire workflow but also offers customizable LeXDual Panel designs for target regions, as well as comprehensive bioinformatics analysis solutions. This fast, simple, user-friendly, and accurate end-to-end solution is designed to better support multi-omics research.

LeXPrep DNA Library Preparation Kit v2 is designed for the preparation of high-quality libraries from double-stranded DNA (dsDNA) on Illumina® and MGI platforms. This A-T ligation-based kit offers a stable and efficient library preparation solution for applications including whole genome sequencing of 1–500 ng DNA and hybridization capture based targeted sequencing. It has improved the library conversion efficiency and better tolerance for low-quality samples. Consequently, it can enhance library complexity while maintaining high fidelity. This kit is fully optimized for size selection with the following the Unique Dual Index (UDI) adapters: LeXPrep Universal Stubby Adapter (UDI) Module and LeXPrep Universal Adapter (MDI) Module (for MGI), as well as the Molecular Identifier adapters: LeXPrep Adapter Kit (customerized) and LeXPrep BMI Adapter (MDI) Module (for MGI). The UDI adapters effectively reduce index-hopping and sample misassignment on Illumina® and MGI platforms. While the customerized adapters, facilitate ultralow-frequency mutation analysis in tumor cells and cfDNA in plasma.

LeXPrep Epicut Module is a methylation-sensitive restriction enzyme module, specifically sensitive to CpG sites in mammals. The stability and cleavage specificity of this module have been systematically validated, ensuring consistent performance.

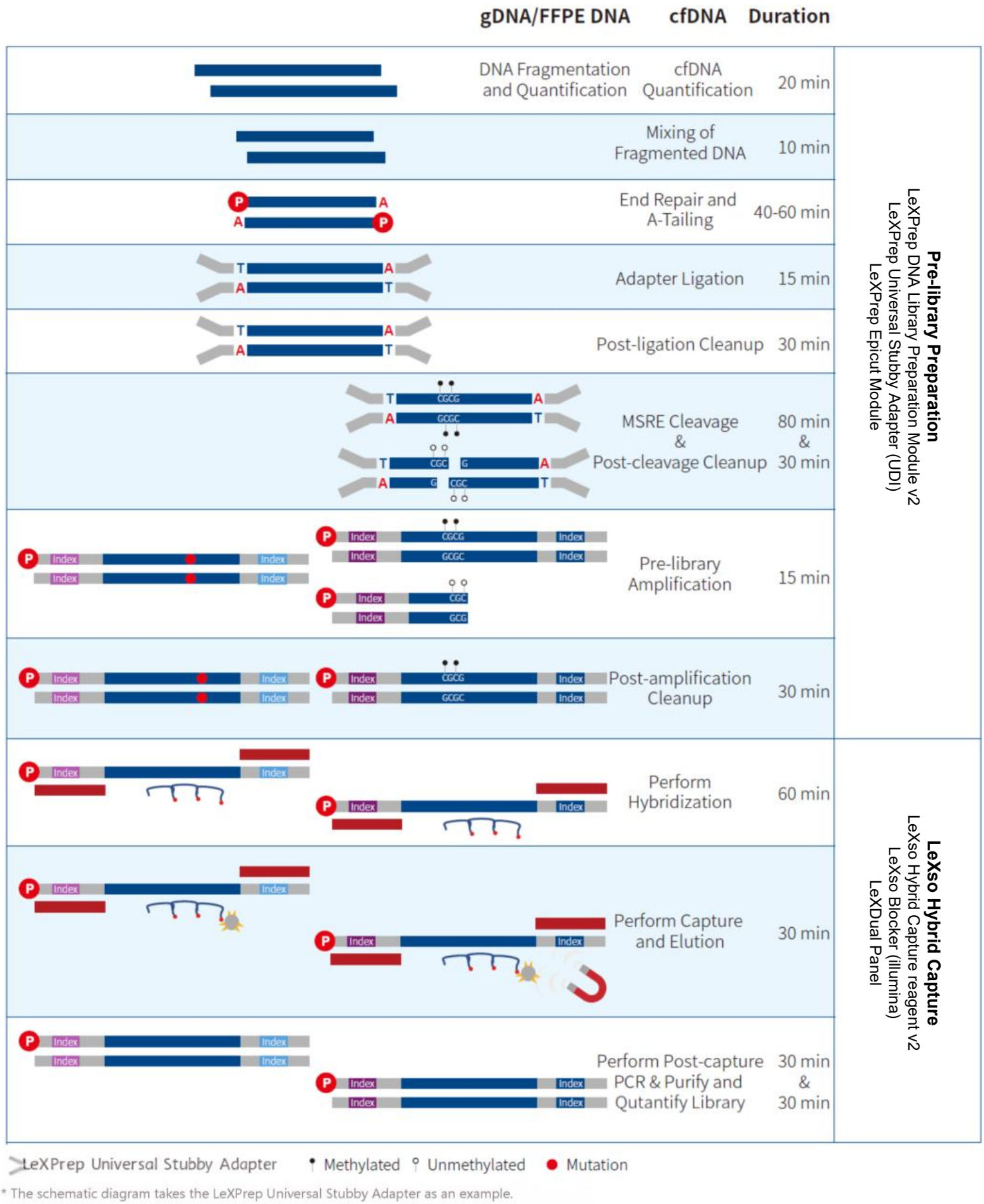
LeXso Hybrid Capture Reagents v2 is designed for targeted enrichment of small Panel and hybrid capture of various types of pre-libraries, integrated with upgraded and optimized hybrid capture and elution processes, and equipped with LeXDual Panel designed based on innovative protocols, which can complete the whole process of captured library preparation in same day.

LeXDual Panel is a commercial or customized Panel developed by LexigenBio, based on proprietary intellectual property. It features a unique LeXso probe design scheme, specifically optimized for the synchronous detection of DNA methylation and mutation. This panel includes fixed probes designed to normalize the depth of MSRE sites and evaluate MSRE cleavage efficiency. The panel is able to quickly find the binding position in the reaction system and further stabilize after multiple probes bind to the target region.

Feature

- **Streamlined Multi-omics Analysis:** Achieve multi-omics analysis using just one sample, one hybridization reaction, and one set of capture probes.
- **Maximized Data Utilization:** Preserve the full diversity of the original sequence without the need for conversion, enhancing overall data utilization.
- **Fast and Convenient:** Simplified and rapid workflow allows for the efficient simultaneous detection of methylation and mutation signals within same day.
- **Enhanced Sensitivity:** Increase the detection sensitivity of low-abundance methylation and ultra-low-frequency mutation signals.

Workflow



* The schematic diagram takes the LeXPrep Universal Stubby Adapter as an example.

Performance

Stable and High Cleavage Efficiency

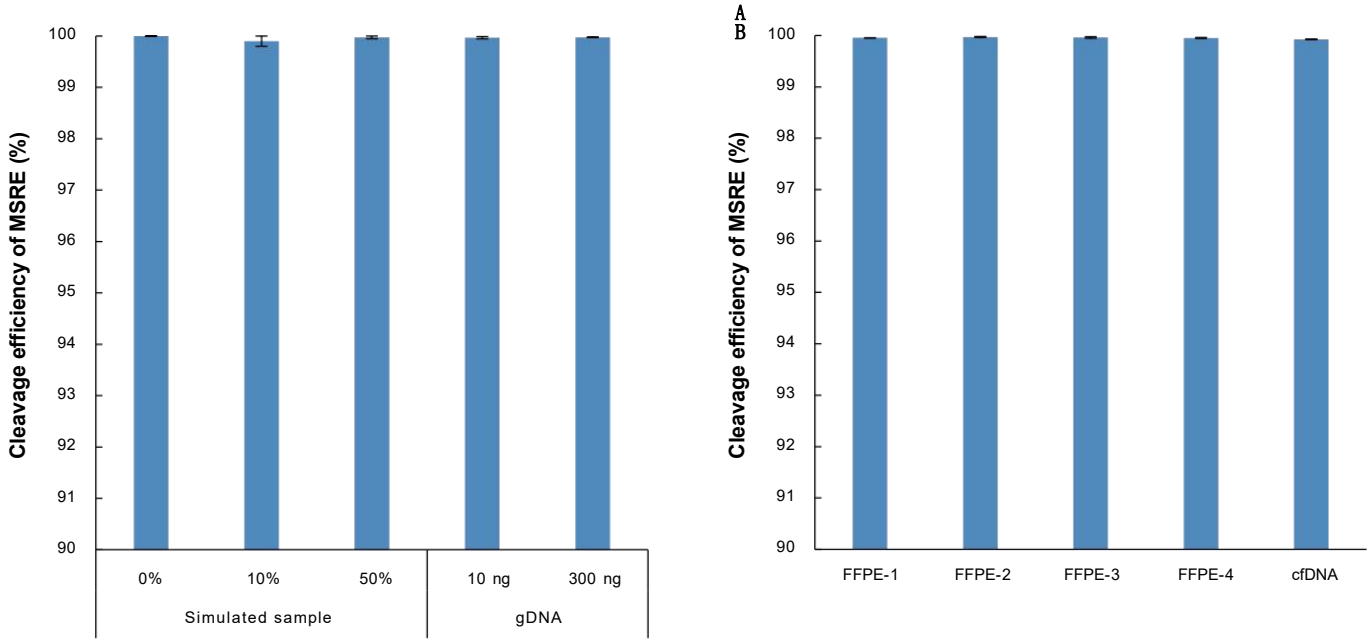


Figure 1. LeXDual can stably and efficiently cut the unmethylated sites across different sample types. A. Simulated samples with varying methylation levels and different initial input amounts of human genomic DNA standard (Promega, G1471); **B.** Clinical FFPE and cfDNA samples.

Note: Samples were simulated DNA by using human 100% Methylated DNA standard (zymo, D5014-2) positive control and 0% Methylated DNA standard (zymo, D5014-1) negative control to mimic different methylation levels (0%, 10%, and 50%). The initial input amounts for both simulated samples with different methylation levels and clinical FFPE and cfDNA samples were 10 ng.

Fully Preserve Original Sequence Information and Enhance Effective Coverage Depth in Methylation Detection

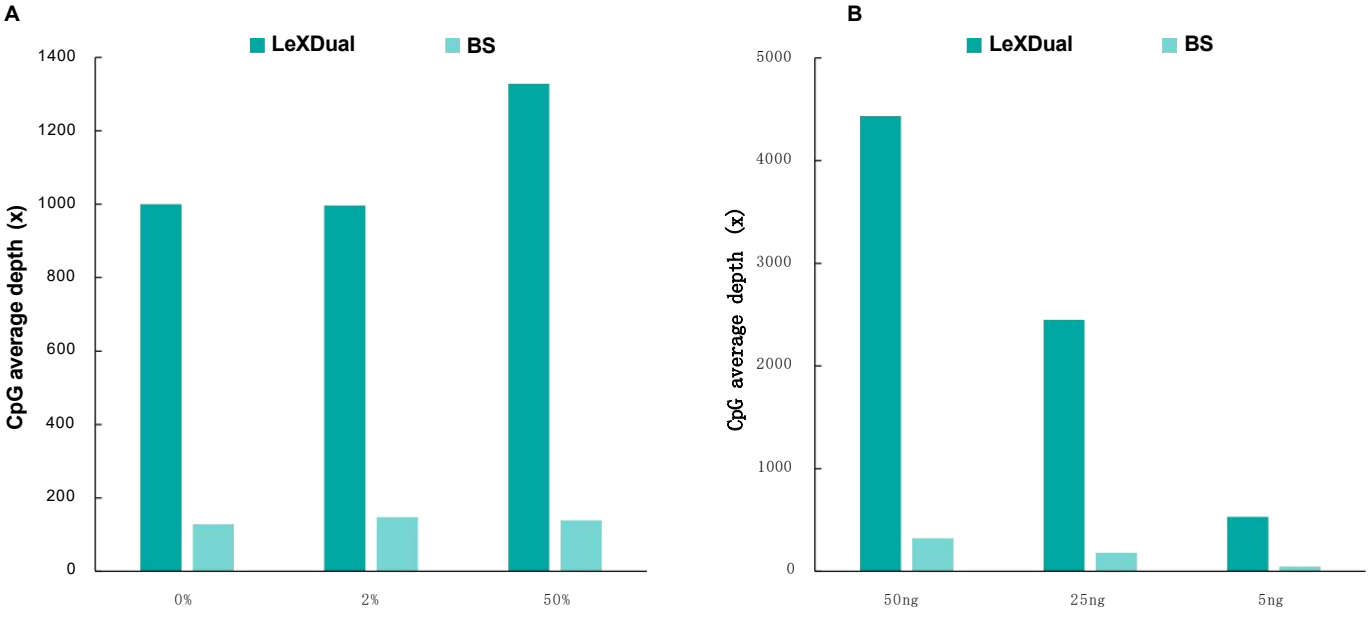


Figure 2. LeXDual can increase the effective coverage depth of CpG sites several times that of the BS treatment. A. Average coverage depth of 13 CpG sites in simulated samples with different methylation levels at the initial inputs of 10 ng and **B.** simulated samples with a methylation level of 5% at different initial input amounts. The simulated samples were processed to hybridization capture using the LeXDual Comprehensive Solution and the LeXso targeted methylation capture solution (BS), respectively. The sequencing mode was Illumina® Novaseq 6000, PE150; 1 M reads pair were randomly selected for data analysis.

Accurate Quantification of Methylation Levels, Enhanced Detection Stability

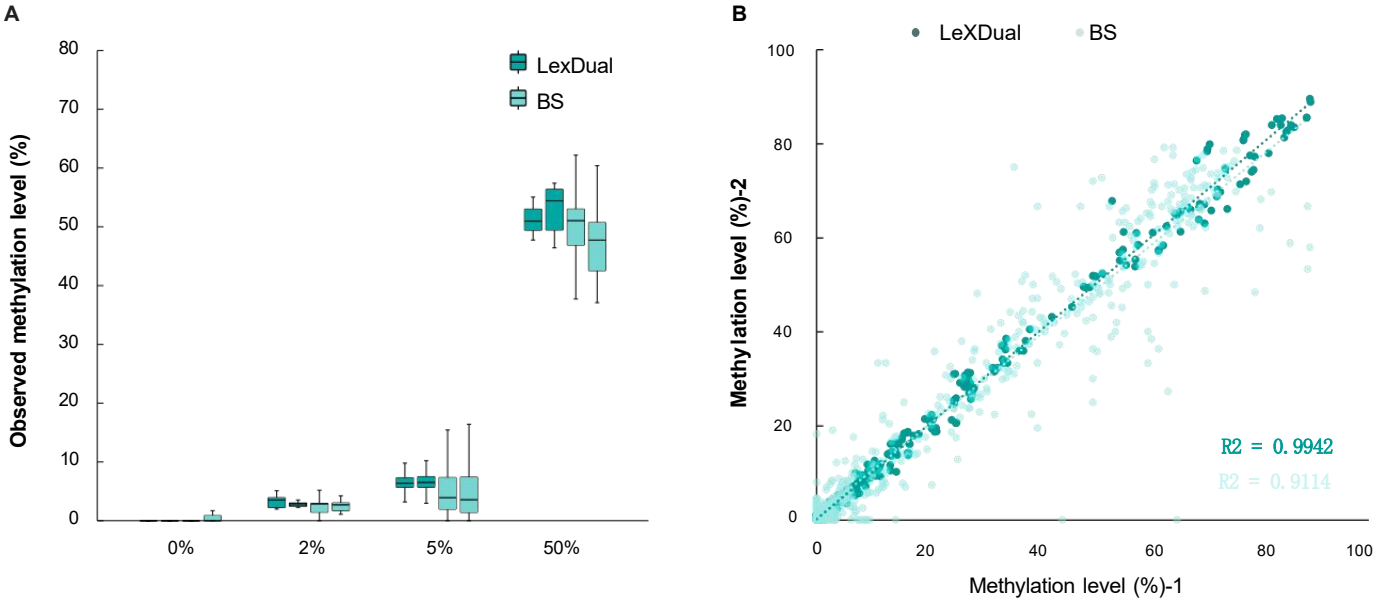
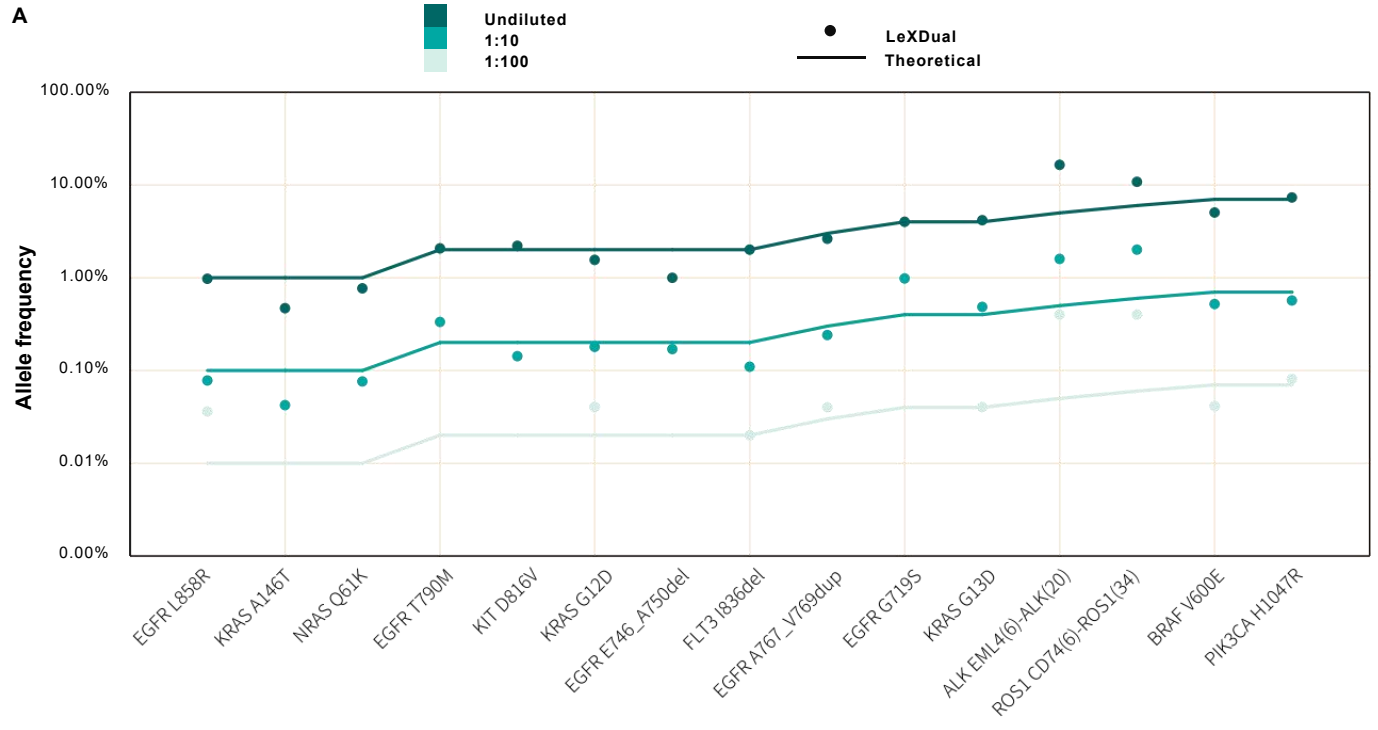


Figure 3. Consistency of detection values obtained with the LeXDual: A. Detection values compared to theoretical values for samples with different methylation levels, and **B.** Consistency between two measurements of one randomly selected clinical colorectal cancer tissue sample. 1 M reads pair were randomly selected for data analysis.

Note: Traditional BS utilized LeXPrep Hybrid Capture Reagents (overnight hybridization) for capture.

Enhanced Sensitivity for Detecting Low-abundance Methylation and Ultra-low-frequency Mutation Signal

Samples were derived from Pancancer Light 800 gDNA Reference Standard (Genewell, GW-OGTM800) (Undiluted) mixed with human genome demethylation control (LDT Bioscience, LDT-600C) at various ratios, followed by 10-fold (1:10) and 100-fold (1:100) dilutions. This created three concentration gradients to simulate different levels of mutation frequencies and methylation states.



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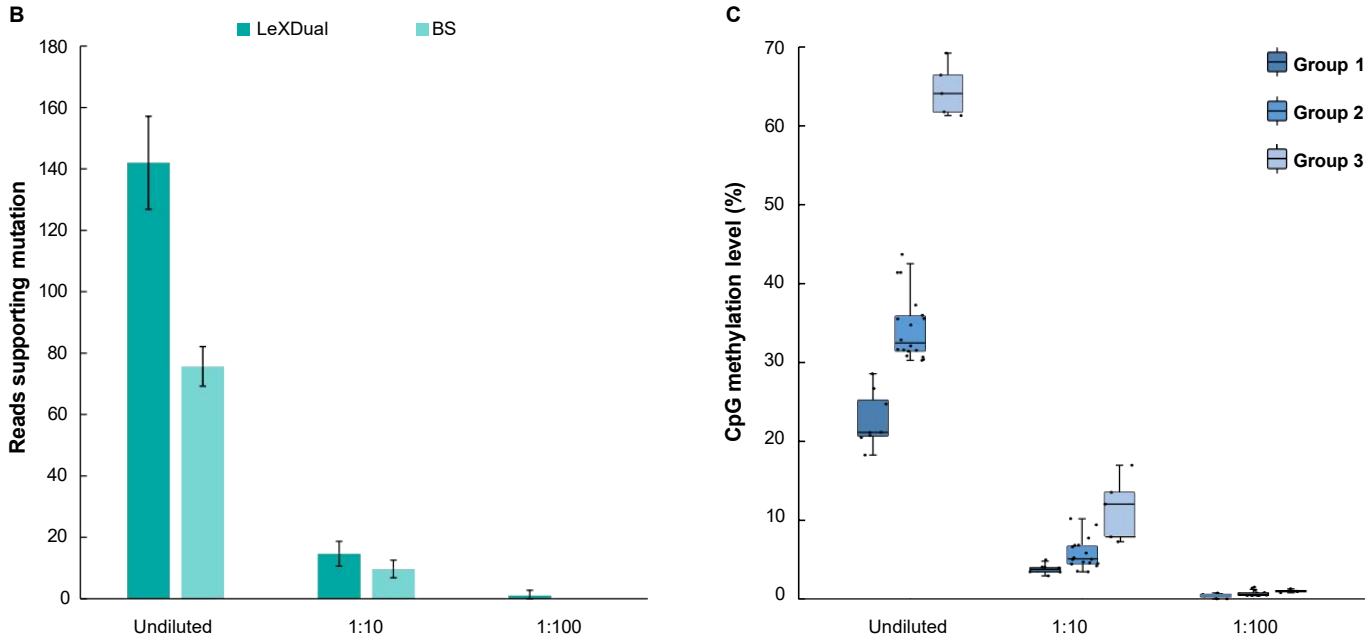

Figure 4. Performance of LeXDual for synchronous detection of ultra-low-frequency mutation and low-abundance methylation. A total of 80 ng of simulated samples were analyzed using LeXDual with Custom Panel (< 5 Kb, covering 31 CpG sites) for hybrid capture. 3 Gb were randomly selected for data analysis. **A.** Mutation frequencies at different sites corresponded closely with the nominal frequencies of the standard [pre-libraries containing molecular identifier, with analytical filtering criteria set to duplex consensus sequences (DCS211)]; **B.** Average number of unique reads supporting mutation detection (DCS211); **C.** Methylation levels of different simulated samples.

Effectively Differentiating Between Tumor and Healthy Population

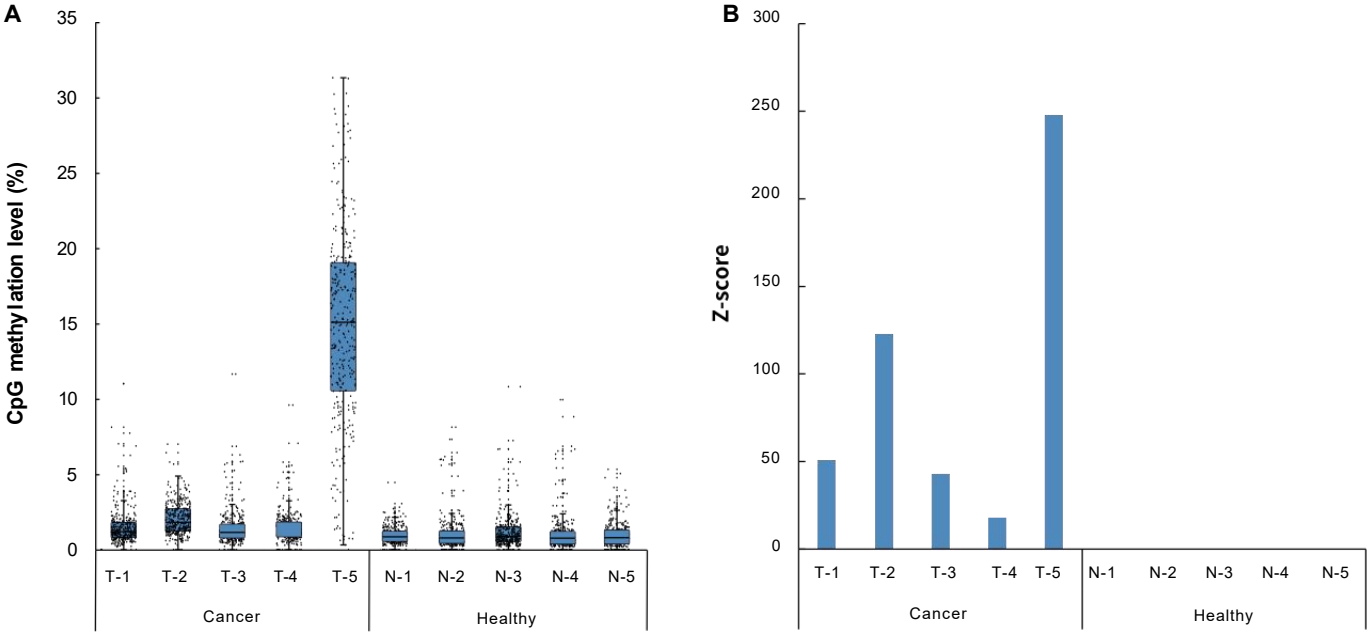

Figure 5. Performance of LeXDual applied to cfDNA detection in clinical colorectal cancer patients and healthy individuals. A. Methylation levels at CpG sites within the target regions; **B.** Z-score analysis.

Note: The initial input amount was 10 ng. T: Plasma cfDNA from clinical colorectal cancer patients, with T-1 to T-4 representing early-stage patients and T-5 representing a late-stage patient; N: Plasma cfDNA from healthy individuals.

Ordering Information

Type		Product	Detail	#Catalog
Pre-libary Preparation	Lib Prep Module	LeXPrep DNA Library Preparation Module v2, 24 rxn	24 rxn	LX02421
		LeXPrep DNA Library Preparation Module v2, 96 rxn	96 rxn	LX02422
	Adapter Module	LeXPrep Universal Stubby Adapter (UDI) Module series	24/96/1152 rxn	LX03240
		LeXPrep Adapter Kit (customized) series	24/96 rxn	LX13111
		LeXPrep Universal Adapter (MDI) Module (for MGI) series	24/96/1152 rxn	LX03711
		LeXPrep BMI Adapter (MDI) Module (for MGI) series	24/96 rxn	LX03911
	Epi Module	LeXPrep Epicut Module, 24 rxn	24 rxn	LX02811
	λ DNA Control	UnMethylated Lambda DNA Control, 30 ng	30 ng	LX05501
Hybrid Capture	Blocker	LeXso Blockers (for Illumina®), 16 rxn	16 rxn	LX16102
		LeXso Blockers (for Illumina®), 96 rxn	96 rxn	LX16101
		LeXso Blockers (for MGI, DI), 16 rxn	16 rxn	LX16212
		LeXso Blockers (for MGI, DI), 96 rxn	96 rxn	LX16211
	Hybrid Capture Reagents	LeXso Hybrid Capture Reagents v2, 16 rxn	16 rxn	LX15202
		LeXso Hybrid Capture Reagents v2, 96 rxn	96 rxn	LX15201
	Panel	LeXDual Beta Panel, 6 rxn	6 rxn	LX11340

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