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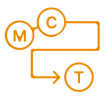
DATA SHEET

Illumina 5-Base DNA Prep with Enrichment

A single assay for simultaneous targeted detection of genomic variants and methylation events



High-sensitivity detection of methylation events using novel chemistry

Two-in-one assay features a streamlined workflow and easy analysis

Flexible customization for targeted genome and methylome insights

High-sensitivity multiomic detection

DNA is inherently multiomic, holding both genetic and epigenetic molecular information. Beyond the sequence of adenine (A), thymine (T), guanine (G), and cytosine (C), there are modified bases such as 5-methylcytosine (5mC) that help direct gene expression (Figure 1). Detecting both genomic variation and DNA methylation can reveal hidden mechanisms of health and disease. Studying the genome and methylome typically requires separate assays and data analysis steps. Illumina 5-Base DNA Prep with Enrichment leverages a novel enzymatic method and optimized analysis for detection of five bases (A, T, G, C, and 5mC) from the same sample, in the same assay, using the same sequencing reads. With hybrid–capture enrichment and custom-designed panels, users can focus on specific targets of interest, enabling cost-effective, deep coverage with high-sensitivity multiomic detection.

Streamlined, flexible workflow

Illumina 5-Base DNA Prep with Enrichment enables targeted DNA sequencing and methylation sequencing with one easy-to-use assay (Figure 2). This single-vendor solution provides a streamlined library-to-analysis workflow that can be completed in less than three days. Illumina 5-Base DNA Prep with Enrichment is compatible with cell-free DNA (cfDNA) and genomic DNA (gDNA) from blood, cell lines, or fresh frozen tissue. Optimized enrichment library prep, which includes a simple, one-step 5mC-to-T base conversion, requires minimal touchpoints and is completed in fewer than 11 hours (Table 1, Figure 3, Figure 4).* Illumina 5-Base DNA Prep with Enrichment uses custom hybrid–capture probe panels for targeted studies. The assay supports four-plex enrichment and up to 384 unique dual indexes. Sequence libraries on the NextSeq™ 2000 System, NovaSeq™ 6000 System, NovaSeq 6000Dx Instrument (RUO mode), or the NovaSeq X Series, followed by simplified data analysis. An integrated DRAGEN™ pipeline generates a dual readout (Figure 5, Figure 6).

* Based on processing 24 samples manually; includes DNA shearing (if applicable), and library quantification. Normalization and pooling times are not included.

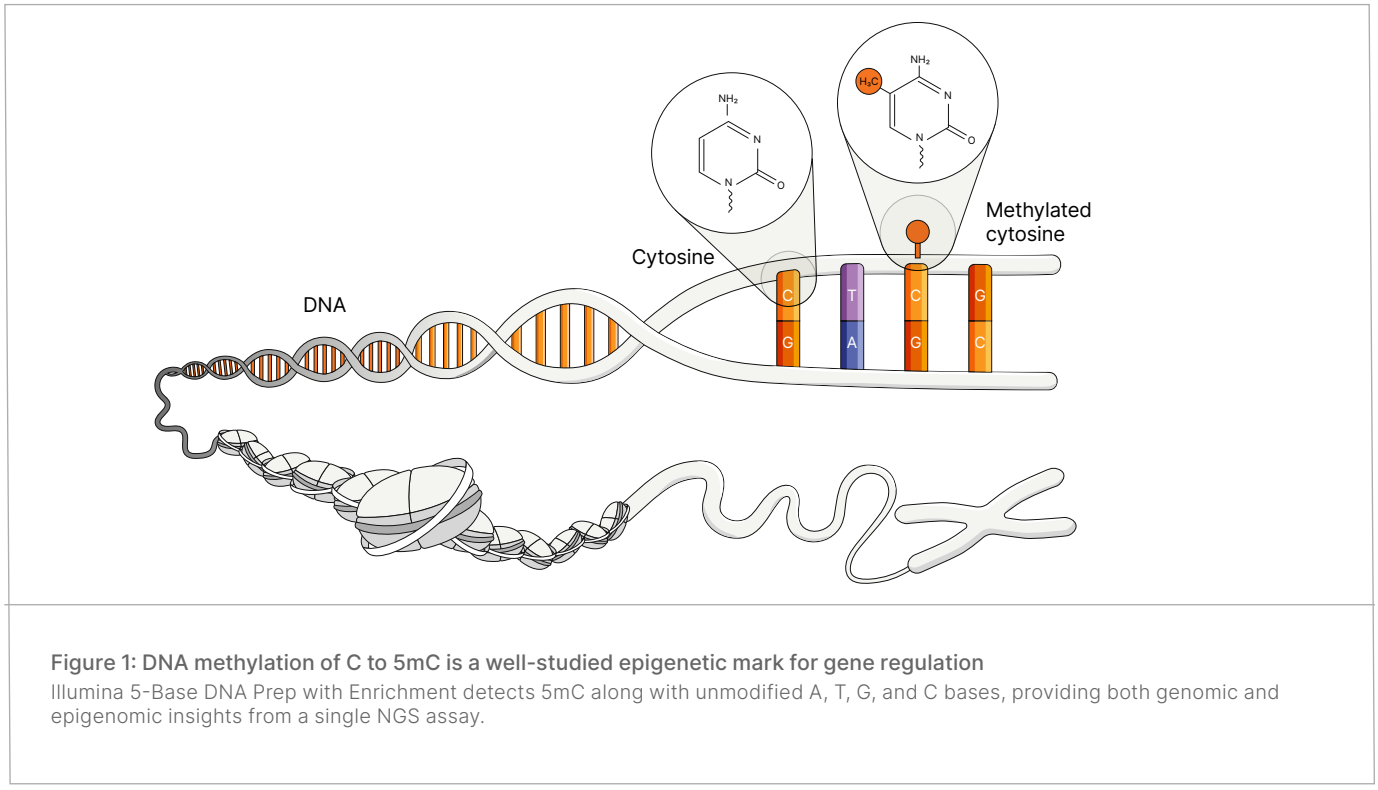


Figure 1: DNA methylation of C to 5mC is a well-studied epigenetic mark for gene regulation
Illumina 5-Base DNA Prep with Enrichment detects 5mC along with unmodified A, T, G, and C bases, providing both genomic and epigenomic insights from a single NGS assay.

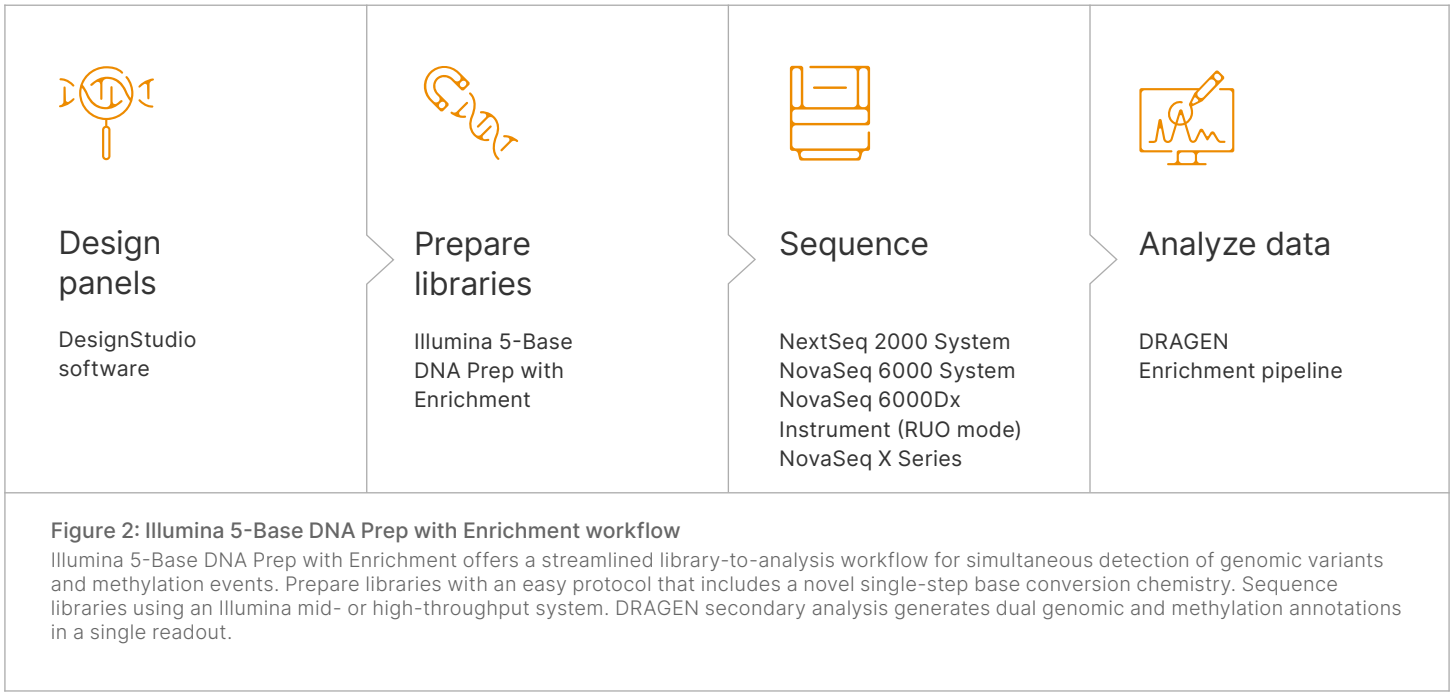


Figure 2: Illumina 5-Base DNA Prep with Enrichment workflow
Illumina 5-Base DNA Prep with Enrichment offers a streamlined library-to-analysis workflow for simultaneous detection of genomic variants and methylation events. Prepare libraries with an easy protocol that includes a novel single-step base conversion chemistry. Sequence libraries using an Illumina mid- or high-throughput system. DRAGEN secondary analysis generates dual genomic and methylation annotations in a single readout.

Table 1: Illumina 5-Base DNA Prep with Enrichment parameters

	Input	Library prep time[a]
Genomic DNA	50–100 ng	< 11 hr
Cell-free DNA	1–20 ng	< 11 hr

a. Based on processing 24 samples manually; includes DNA shearing (if applicable), and library quantification. Normalization and pooling times are not included.

Novel chemistry for direct conversion of 5-methylcytosine to thymine

Illumina 5-Base DNA Prep with Enrichment is a fundamentally different approach to targeted genome and methylome analysis. Traditional methods for detecting DNA methylation use bisulfite treatment or enzymes to convert unmethylated cytosine to thymine (Figure 3B). Because most cytosines in the genome are unmodified, this approach greatly reduces nucleotide diversity, making sequencing reads harder to align. Illumina 5-Base DNA Prep with Enrichment uses a novel engineered enzyme to directly convert only 5mC to thymine in a single incubation step during library preparation (Figure 3A). Illumina 5-base chemistry preserves library complexity and ensures high data alignment rate, increasing the sensitivity of detection.

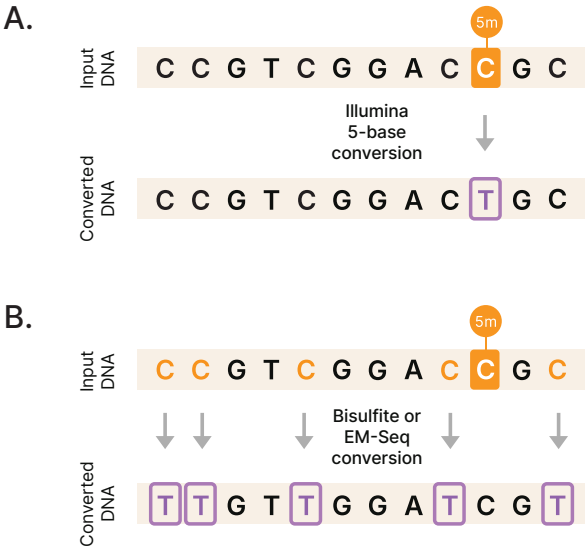


Figure 3: Novel chemistry directly converts 5mC to T in a single step
(A) Illumina 5-Base DNA Prep with Enrichment uses a one-step enzymatic process to convert only 5mC to T, yielding greater nucleotide diversity than (B) traditional bisulfite sequencing or on-market enzymatic methylation sequencing (EM-Seq), in which unmethylated C are converted to T.

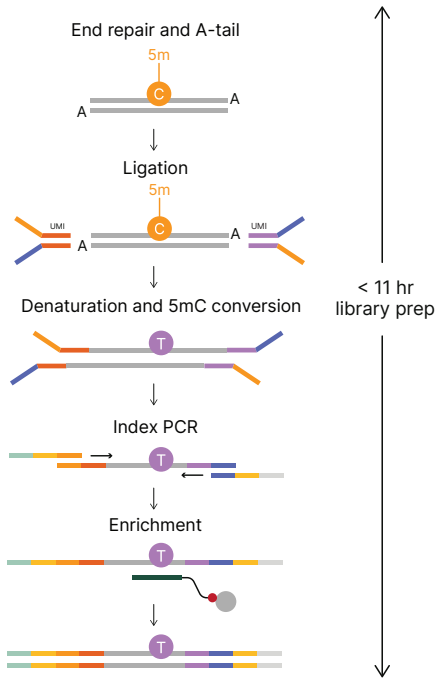


Figure 4: Illumina 5-Base DNA Prep with Enrichment uses an optimized ligation-based library preparation workflow

Single readout with combined genome and methylome data

Integrated DRAGEN secondary analysis provides accurate annotation of both methylation and genomic variants in a single readout (Figure 5, Figure 6). Novel methylation-aware algorithms leverage the complementary strand sequence to discern between a thymine indicating a methylation event and a thymine that represents a single nucleotide variant (SNV) (Figure 5). Integrated unique molecular identifiers (UMIs) help provide confident single-molecule resolution of methylation status. Duplex UMI collapsing improves technical reproducibility for detecting small methylome differences and allows sensitive interrogation of asymetric methylation across cytosine-guanine dinucleotide (CpG) sites (Figure 5).

Illumina 5-base methylation reporting features are available within the DRAGEN Enrichment pipeline with an easy checkbox option. Secondary analysis can be performed via BaseSpace™ Sequence Hub or Illumina Connected Analytics cloud platforms, or on a DRAGEN server.

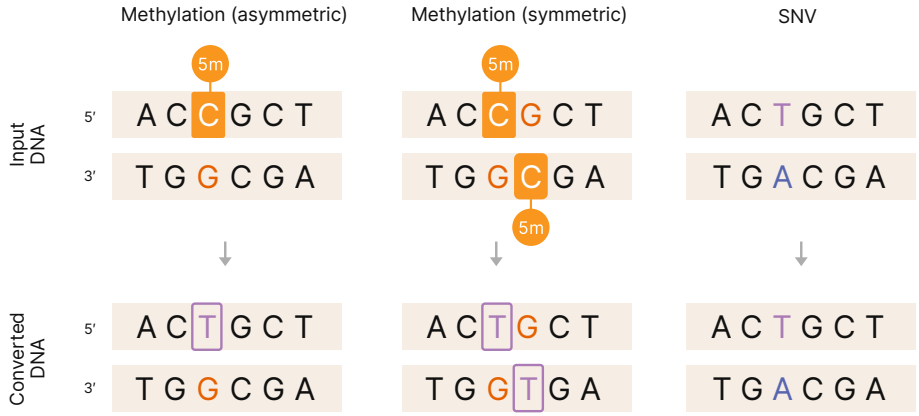


Figure 5: DRAGEN algorithms distinguish methylation events from SNVs

Innovative DRAGEN algorithms leverage the complementary strand sequence to accurately discern between methylation and small variant calls in the same read. For 5mC converted to T, the complementary base will be G, whereas for a C-to-T genomic variant, the complementary base will be A. Duplex UMI collapsing further enables single-molecule resolution to detect symmetric and asymmetric methylation at CpG sites.

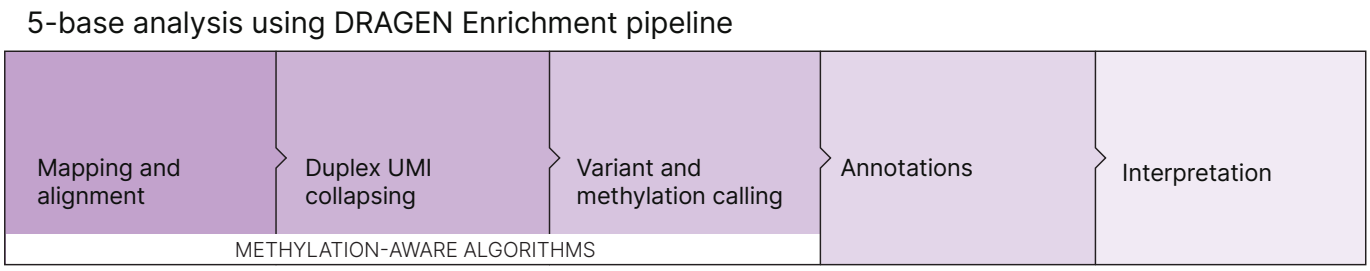


Figure 6: DRAGEN Enrichment pipeline with integrated methylation-aware algorithms enables accurate annotation of both methylation and genomic variants in a single readout

Illumina 5-base secondary analysis is available within the DRAGEN Enrichment pipeline with an easy checkbox option to activate methylation reporting. UMI, unique molecular identifiers.

Customization to focus on regions of interest

Illumina 5-Base DNA Prep with Enrichment requires panels specifically designed for the 5-base assay. Panel design is key for optimal performance and efficient capture of both methylated and unmethylated targets.† To focus sequencing on regions of interest, Illumina Custom Enrichment Panel v2, in a broad range of panel sizes, can be designed and ordered using DesignStudio™ software. The specialized design algorithm accommodates the 5mC-to-T base conversion unique to the Illumina 5-base solution.† DesignStudio software supports design of custom panels from target gene lists or genomic coordinates. Content from existing panels can be used as a starting point. The DesignStudio tool also allows users to select regulatory regions, such as promoters, within defined targets.

† Standard enrichment panels do not account for methylated and nonmethylated bases. Panels made for methods that convert unmethylated C, such as bisulfite sequencing, are designed for 3-base nucleotide diversity (Figure 3) and are not compatible with Illumina 5-Base DNA Prep with Enrichment.

High-sensitivity detection

Illumina 5-Base DNA Prep with Enrichment is optimized to enable sensitive methylation and variant detection for multiple applications. Illumina 5-Base DNA Prep with Enrichment shows high coverage uniformity across targets of interest for both large (1 Mb) and small (75 kb) panels (Figure 7, Figure 8). The measured methylation levels are highly accurate across sample types and DNA inputs (Figure 9). Illumina 5-Base DNA Prep with Enrichment also shows unbiased capture of target regions across a range of methylation levels (Figure 10). 5mC conversion is highly selective across a range of sample inputs, as measured by small genome control spike-ins (Figure 11). Intregrated UMIs allow detection of low allele frequency variants from low-input samples with high accuracy (Figure 12).

With complementary workflows, users can scan the whole genome and methylome using Illumina 5-Base DNA Prep for broad discovery, then use those results to guide targeted panel design for deeper sequencing using Illumina 5-Base DNA Prep with Enrichment.

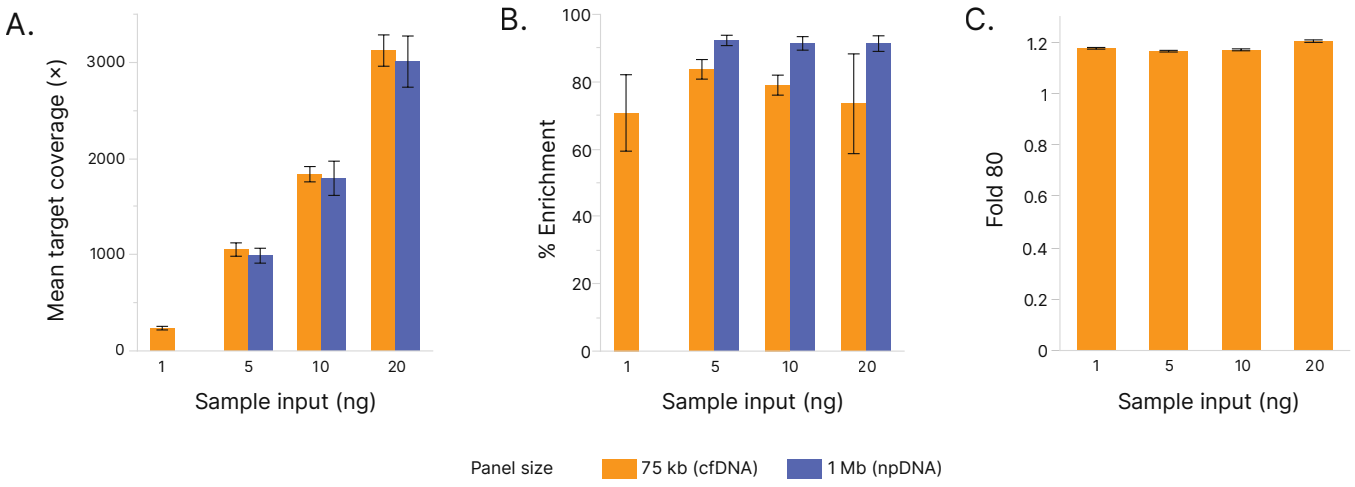


Figure 7: High coverage and uniformity across targets of interest for cfDNA and npDNA

(A) Mean target coverage, (B) percent enrichment, and (C) fold-80 base penalty for Illumina 5-Base DNA Prep with Enrichment libraries prepared from 1–20 ng of cell-free DNA (cfDNA) (orange) and nucleosomal prepared DNA (npDNA) (blue). cfDNA is extracted from serum of healthy donors; npDNA is from NA12878 and NA12877 cell lines (Coriell Institute for Medical Research). Libraries were enriched using Illumina Custom Enrichment Panel v2 (Illumina, Catalog no. 20073953) designed for Illumina 5-Base DNA Prep with Enrichment. cfDNA libraries were enriched with a 75-kb custom panel, sequenced on the NextSeq 2000 System, and data was downsampled to 25M clusters (50M paired-end reads). npDNA libraries were enriched with a 1-Mb custom panel, sequenced on the NovaSeq X System, and data was downsampled to 200M clusters (400M paired-end reads). Secondary analysis was performed using DRAGEN Enrichment v4.4.6. Mean target coverage and percent enrichment were determined after UMI collapsing.

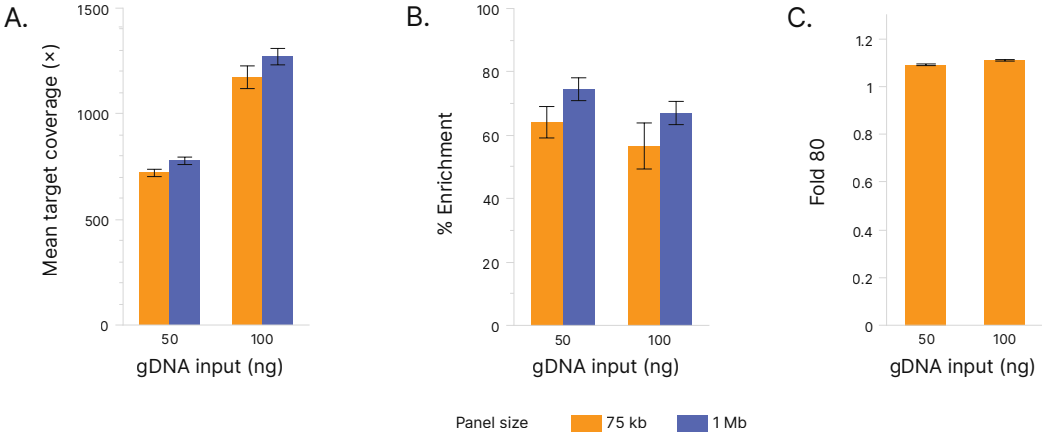


Figure 8: High coverage and uniformity across targets of interest for gDNA

(A) Mean target coverage, (B) percent enrichment, and (C) fold-80 base penalty for Illumina 5-Base DNA Prep with Enrichment libraries prepared from 50 or 100 ng NA12878 genomic DNA (gDNA) spiked with 5% NA12877 gDNA for subsequent variant calling. Libraries were enriched with Illumina Custom Enrichment Panel v2 designed for Illumina 5-Base DNA Prep with Enrichment, targeting 75 kb (orange) or 1 Mb of the genome (blue). 75-kb enriched libraries were sequenced on the NextSeq 2000 System and data was downsampled to 2M clusters (4M paired-end reads). 1-Mb enriched libraries were sequenced on the NovaSeq X System and data was downsampled to 25M clusters (50M paired-end reads). Secondary analysis was performed using DRAGEN Enrichment v4.4.6. Mean target coverage and percent enrichment were determined after UMI collapsing.

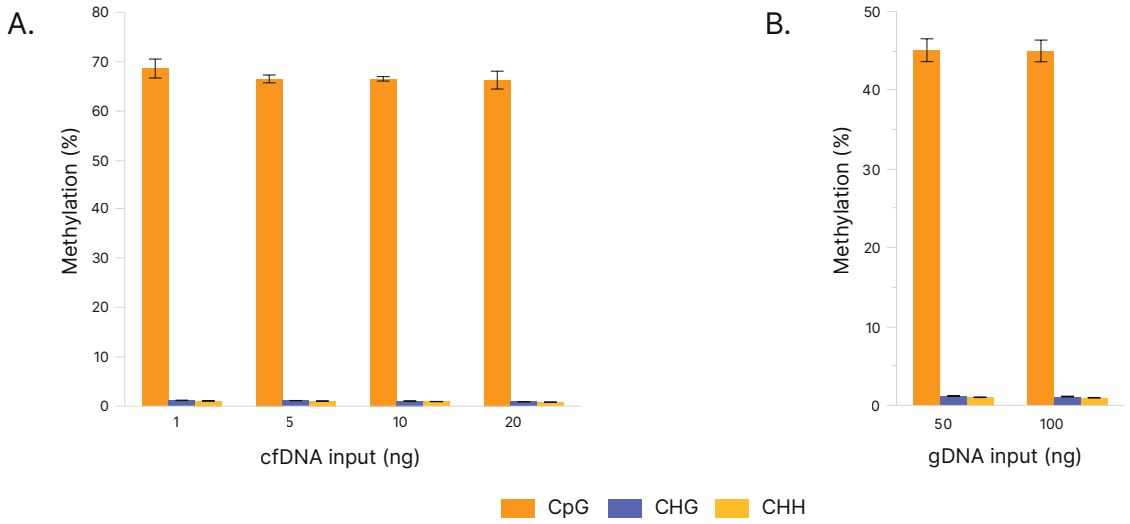


Figure 9: Accurate methylation detection with Illumina 5-Base DNA Prep with Enrichment across sample types and DNA inputs

Percent methylation of cytosines in CpG, CHG, and CHH genomic context. (A) Illumina 5-Base libraries were prepared from 1–20 ng input of cfDNA extracted from serum of healthy donors or (B) 50 and 100 ng gDNA from reference human genome sample NA12878 spiked with 5% NA12877. Global average CpG methylation for cfDNA and reference gDNA sample types are shown for CpG sites targeted by a 75-kb panel. Very low levels of background CHG and CHH methylation are observed as expected. Libraries were sequenced on the NextSeq 2000 or NovaSeq X Systems. Secondary data analysis was performed with DRAGEN Enrichment v4.4.6.

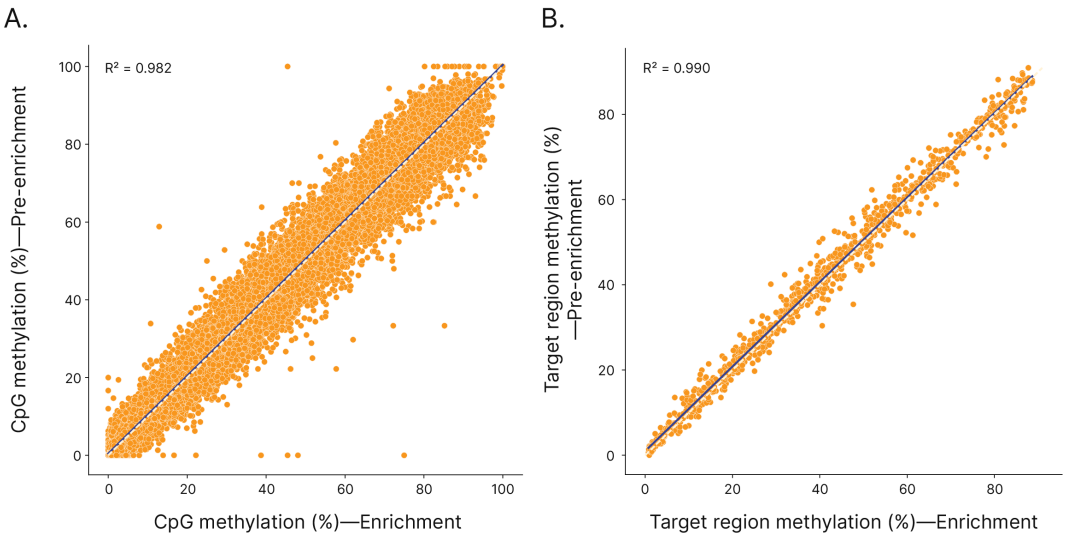


Figure 10: Unbiased capture of target regions across a range of methylation levels

Strong correlation between enriched and pre-enriched libraries for (A) the measured methylation level for all targeted CpG positions (R2 = 0.982, slope = 1.00), and (B) the average methylation levels across target regions (R2 =0.990, slope = 0.99). Libraries were prepared from 10 ng npDNA and enriched with a 1-Mb Illumina Custom Enrichment Panel v2 designed for Illumina 5-Base DNA Prep with Enrichment. Enriched libraries and the pre-enriched libraries were sequenced on the NovaSeq X System. Secondary data analysis was performed with DRAGEN Enrichment v4.4.6.

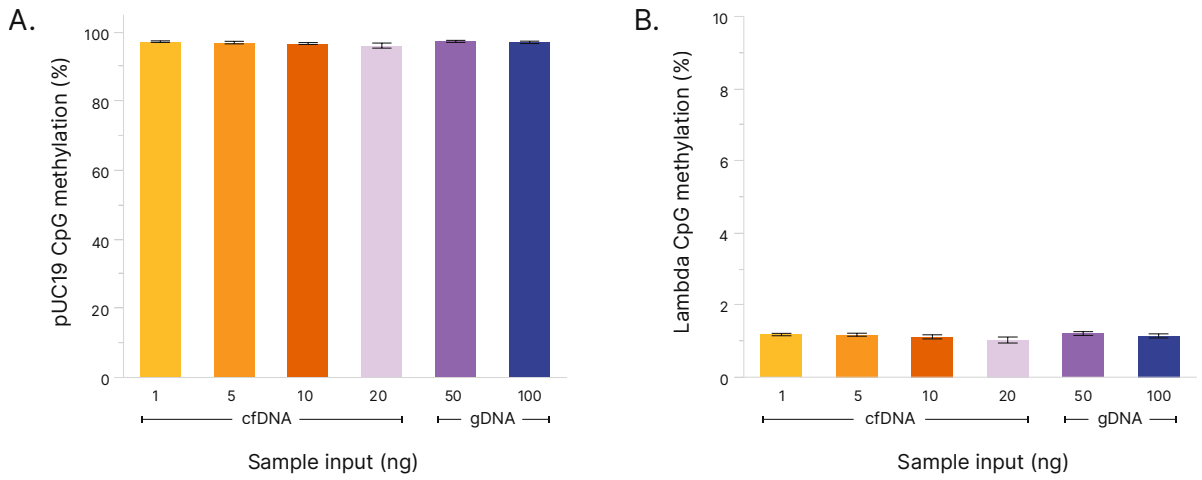


Figure 11: Selective methylation conversion with Illumina 5-Base DNA Prep

High selectivity and consistent methylation conversion across a range of sample input amounts and sample types supports a range of applications. Small genome controls (A) methylated pUC and (B) unmethylated lambda are included in the kit and can be spiked in along with sample of interest for methylation conversion quality control (QC). Input amounts are 1–20 ng cfDNA from healthy donors and 50–100 ng gDNA from human reference sample NA12878 spiked with 5% NA12877 gDNA. Secondary data analysis was performed with DRAGEN Enrichment v4.4.6.

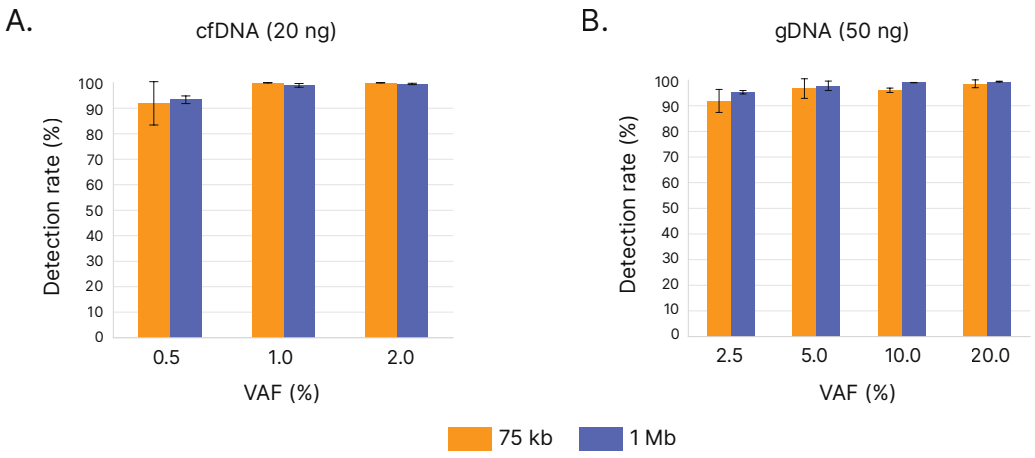


Figure 12: Illumina 5-base DNA Prep with enrichment detects low frequency variants with high accuracy

Variant detection rates for (A) libraries prepared from mixtures of npDNA from NA12877 and NA12878 to mimic cfDNA with variants at 0.5%, 1%, and 2% variant allele frequencies (VAF) and (B) libraries prepared from mixtures of gDNA from NA12877 and NA12878 to obtain truth variants at 2.5%, 5%, 10%, and 20% VAF. Libraries were enriched using Illumina Custom Enrichment Panel v2 designed for Illumina 5-Base DNA Prep with Enrichment, targeting 75 kb (orange) or 1 Mb of the genome (blue). Mixed npDNA libraries enriched with the 75-kb panel were sequenced on the NextSeq 2000 System and data was downsampled to 25M clusters (50M paired-end reads). Mixed npDNA libraries enriched with the 1-Mb panel were sequenced on the NovaSeq X System and data was downsampled to 200M clusters (400M paired-end reads). Mixed gDNA libraries enriched with the 75-kb panel were sequenced on the NextSeq 2000 System and data was downsampled to 2M clusters (4M paired-end reads). Mixed gDNA libraries enriched with the 1-Mb panel were sequenced on the NovaSeq X System and data was downsampled to 25M clusters (50M paired-end reads). Variant calling was performed with DRAGEN 4.4.6.

Summary

Illumina 5-Base DNA Prep with Enrichment offers a library-to-analysis solution for simultaneous targeted genome and methylome profiling in one optimized assay. Custom enrichment panel design focuses sequencing on regions of interest. Illumina 5-Base DNA Prep with Enrichment enables high-sensitivity methylation and variant calling from cfDNA and somatic variant calling from genomic DNA to maximize insights from each read.

Learn more →

Illumina 5-Base DNA Prep with Enrichment

Ordering information

Product	Catalog no.
Library prep	
Illumina 5-Base DNA Prep with Enrichment (24 samples)	20140366
Illumina 5-Base DNA Prep with Enrichment (96 samples)	Coming soon
Panels	
Illumina Custom Enrichment Panel v2 (32 µl, 120 bp)	20073953
Illumina Custom Enrichment Panel v2 (384 µl, 120 bp)	20073952
Illumina Custom Enrichment Panel v2 (1536 µl, 120 bp)	20111339
Indexes	
Illumina DNA/RNA UD Indexes Set A, Tagmentation (96 indexes, 96 samples)	20091654
Illumina DNA/RNA UD Indexes Set B, Tagmentation (96 indexes, 96 samples)	20091656
Illumina DNA/RNA UD Indexes Set C, Tagmentation (96 indexes, 96 samples)	20091658
Illumina DNA/RNA UD Indexes Set D, Tagmentation(96 indexes, 96 samples)	20091660
Illumina Unique Dual Indexes, LT (48 indexes, 48 samples)	20098166
Analysis	
Illumina DRAGEN server v4	20051343
Illumina Analytics - 1 iCredit	20042038
Illumina Analytics Starter Package - 1000 iCredits	20042039
Illumina Analytics - 5000 iCredits	20042040
Illumina Analytics - 50,000 iCredits	20042041
Illumina Analytics - 100,000 iCredits	20042042

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