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DATA SHEET

Illumina 5-Base DNA Prep

Detect methylation events and genomic variants in a single whole-genome assay

High-quality data enabled by novel chemistry and analytical methods

Two-for-one assay with easy workflow and analysis

Cost-effective discovery through multiomic insights

Comprehensive multiomic discovery

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 next-generation sequencing (NGS) assays and data analysis steps. Additionally, most NGS methylation profiling methods have tedious, complex workflows.

Illumina 5-Base DNA Prep leverages unique chemistry and optimized analysis algorithms for a fundamentally different approach to genome and methylome interrogation and analysis. A novel enzymatic method allows detection of five bases (A, T, G, C, and 5mC) from a single sample, library preparation, sequencing run, and analysis workflow. Illumina 5-Base DNA Prep provides a high-quality, easy-to-use, cost-efficient assay for simultaneous discovery of genomic variants and methylation events across the whole genome.

Simple and fast workflow

Illumina 5-Base DNA Prep combines whole-genome sequencing (WGS) and methylation sequencing into one easy-to-use workflow (Figure 2). This single-vendor solution provides a streamlined library-to-interpretation workflow with a turnaround time of less than three days—up to 3× faster than alternative NGS methods.[1–5] Illumina 5-Base DNA Prep is compatible with cell-free DNA (cfDNA) and genomic DNA (gDNA) from blood, cell lines, or fresh frozen tissue (Table 1). Optimized library prep, which includes a rapid, one-step 5mC-to-T base conversion, requires minimal touchpoints and is completed in a single day (Table 1, Figure 3, Figure 4).* The 5-base solution is flexible and scalable to support a breadth of research studies on the NovaSeq™ X Series, NovaSeq 6000 System, or NovaSeq 6000Dx Instrument (RUO mode) (Table 2, Table 3). Streamlined secondary analysis with 5-base DRAGEN™ pipelines generates a dual readout in as little as an hour† for a 30× genome (Figure 5, Figure 6). Illumina Connected Multiomics deepens analysis with industry-proven statistical methods and information-rich, clear visualizations (Figure 7).

* Less than 6 hours for cfDNA library preparation workflow, less than 8 hours for gDNA library preparation workflow (includes fragmentation time). Time does not include quality control (QC) or sequencing set up.
† Single sample DRAGEN Germline pipeline on a DRAGEN server.

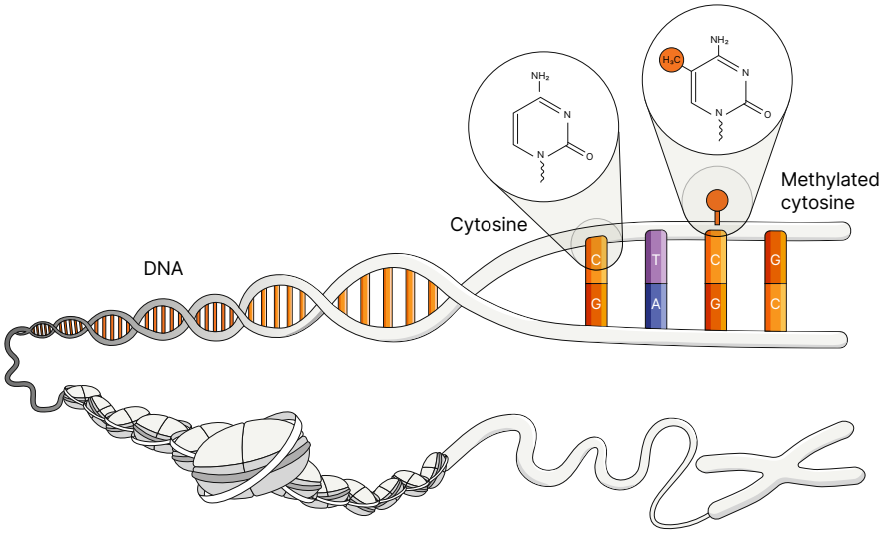


Figure 1: DNA methylation of C to 5mC is a well-studied epigenetic mark for gene regulation
Illumina 5-Base DNA Prep detects 5mC along with unmodified A, T, G, and C bases, providing both genomic and epigenomic insights from a single NGS assay.

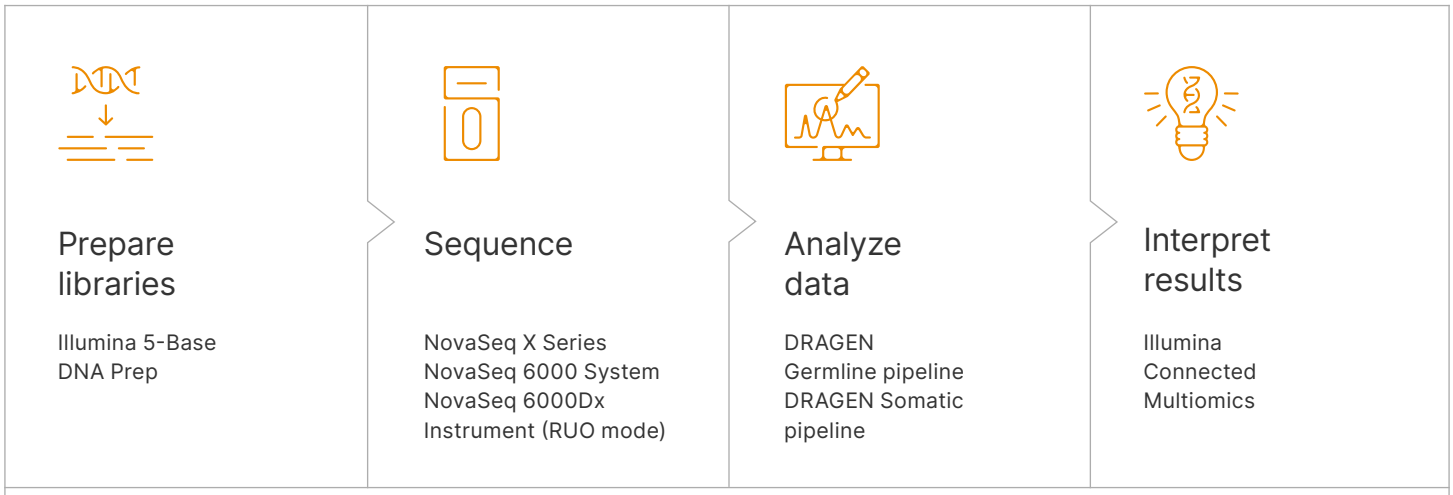


Figure 2: Illumina 5-Base DNA Prep workflow

Illumina 5-Base DNA Prep offers a streamlined library-to-interpretation workflow for simultaneous detection of genomic variants and methylation events. Prepare libraries in less than a day with an easy protocol that includes a novel single-step base conversion chemistry, then sequence using an Illumina high-throughput system. DRAGEN secondary analysis generates dual genomic and epigenomic annotations in a single readout in less than an hour for a 30× genome. Illumina Connected Multiomics provides clear visualizations and analysis tools to simplify data interpretation.

Table 1: Illumina 5-Base DNA library prep parameters

Sample type	Input quantity	Library prep time
Genomic DNA	50–100 ng	< 8 hr[a]
Cell-free DNA	1–20 ng	< 6 hr

a. Includes fragmentation time.

Table 2: Sample throughput for germline variant calling and methylation applications

NovaSeq X flow cell	25B	10B	1.5B
No. samples per flow cell[a]	48	18	3

a. 2 × 151 bp runs and ≥ 500M clusters for high-accuracy SNV and indel germline variant calling.

Table 3: Sequencing coverage recommendations for different applications with Illumina 5-Base DNA Prep

Use case	Sequencing coverage	DRAGEN secondary analysis pipeline
Germline 5-base genome	35–40×	Germline
Whole-methylome sequencing	10–35×	Germline
Methylome + tumor-only somatic variant calling	≥ 100×	Somatic
Methylome + tumor–normal somatic variant calling[a]	≥ 100×/≥ 50×	Somatic
Methylome + germline CNV calling[b]	30×	Germline
Methylome + somatic CNV calling[b]	≥ 80×	Somatic
cfDNA methylome/fragmentation profiling	≥ 30×	Somatic

a. Normal coverage should be half of tumor coverage and target ≥ 100× tumor coverage.
b. CNV, copy number variant.

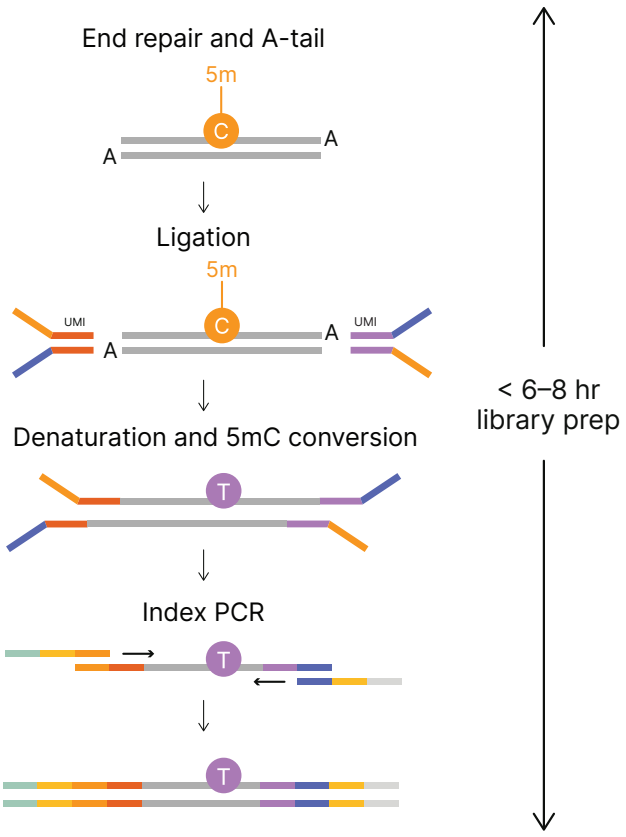


Figure 3: Library preparation steps for Illumina 5-Base DNA Prep

Optimized ligation-based library preparation can be completed in less than one day. Time required is less than six hours for cfDNA workflow, less than eight hours for gDNA workflow (includes fragmentation). Time does not include QC or sequencing set up.

Novel chemistry for direct conversion of 5-methylcytosine to thymine

Traditional methods for detecting DNA methylation use bisulfite treatment or enzymes to convert unmethylated cystosine to thymine (Figure 4). Because the majority of cytosines in the genome are unmodified, this approach greatly reduces nucleotide diversity, making reads harder to sequence and align. Bisulfite treatment can also damage DNA, leaving data gaps. Illumina 5-Base DNA Prep uses a novel engineered enzyme to directly convert only 5mC to T in a single incubation step (Figure 4). The Illumina 5-base method is nondamaging to DNA and retains four-base nucleotide diversity for more efficient alignment, maximizing data from every read (Table 4).

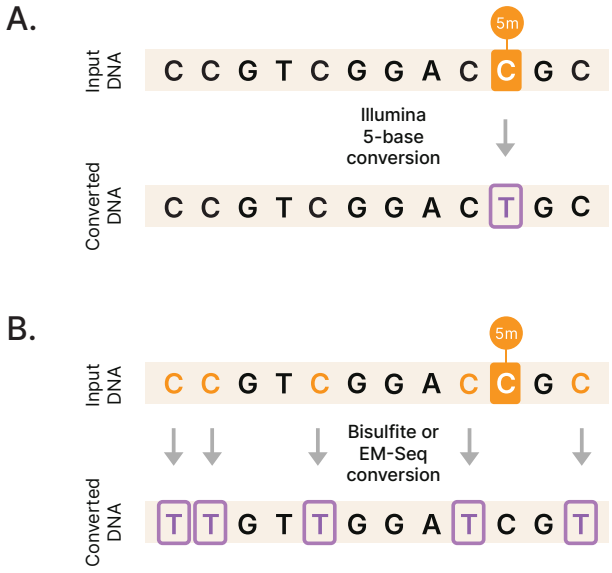


Figure 4: Novel chemistry directly converts 5mC to T in a single enzymatic step

(A) Illumina 5-Base DNA Prep uses a one-step enzymatic process to convert only 5mC to T, yielding greater nucleotide diversity than (B) traditional bisulfite treatment or enzymatic methylation sequencing (EM-Seq), in which unmethylated C are converted to T.

Table 4: Advantages of Illumina 5-Base DNA Prep

Traditional methylation sequencing methods	Illumina 5-base solution
Challenges	Benefits
• Limited mapping efficiency • Low variant detection accuracy • Chemical conversion that damages DNA[a] • Multistep conversion	• High mapping efficiency • High-accuracy variant detection • Enzymatic conversion that is nondamaging to DNA • Single-step conversion

a. For bisulfite conversion NGS methods.

Single readout with combined genome and methylome data

Integrated DRAGEN secondary analysis provides accurate annotation of both methylation and genomic variants in a single data set (Figure 5). Novel 5-base methylation-aware DRAGEN algorithms account for the stranded nature of methylation to discern between a thymine that indicates a methylation event and a thymine that represents a single nucleotide variant (SNV) (Figure 6). Methylation and genome variation are captured down to single-molecule resolution, enabling deep investigations of biological mechanisms.

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

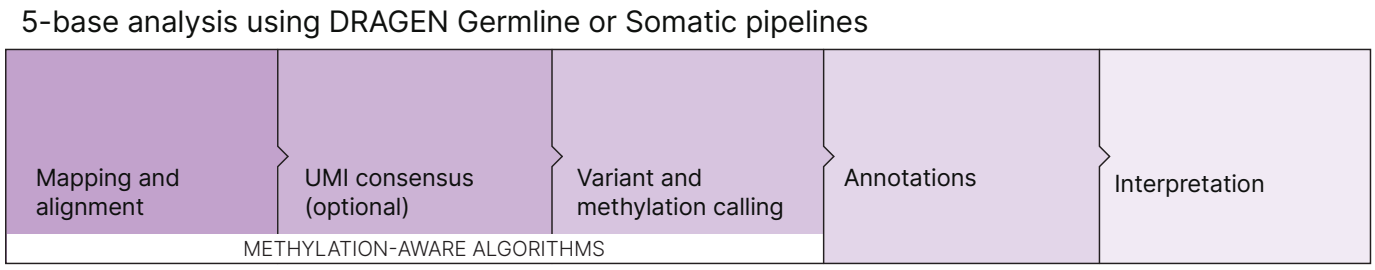


Figure 5: Novel methylation-aware algorithms in DRAGEN Germline or Somatic pipelines
Illumina 5-base secondary analysis is available within the DRAGEN Germline and DRAGEN Somatic pipelines with an easy checkbox option to activate methylation reporting. Streamlined 5-base secondary analysis with DRAGEN pipelines generates a dual readout in as little as an hour for a 30× genome (single sample DRAGEN Germline pipeline on a DRAGEN server). UMI, unique molecular identifiers.

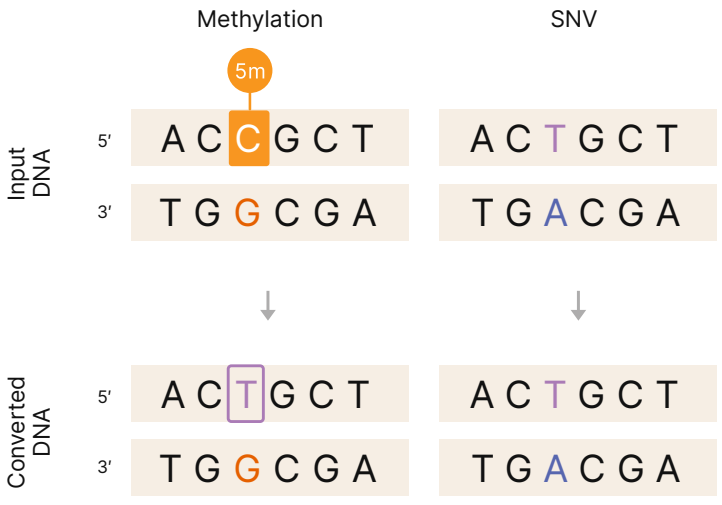


Figure 6: DRAGEN secondary analysis with 5-base methylation-aware algorithms distinguishes methylation events from SNVs

Innovative algorithms leverage the complementary strand sequence to accurately discern between methylation and small variant calls in the same read. For 5mC converted to T (purple box), the complementary base will be G, whereas for a C-to-T genomic variant, the complementary base will be A.

Dive deeper with Illumina Connected Multiomics

The resulting DRAGEN output files can be directly imported into Illumina Connected Multiomics for intuitive data exploration and visualization, including diverse differential analyses (Figure 7). Uncover methylation patterns, group samples, reduce data dimensionality, detect and annotate biomarkers, and link findings to biological functions.

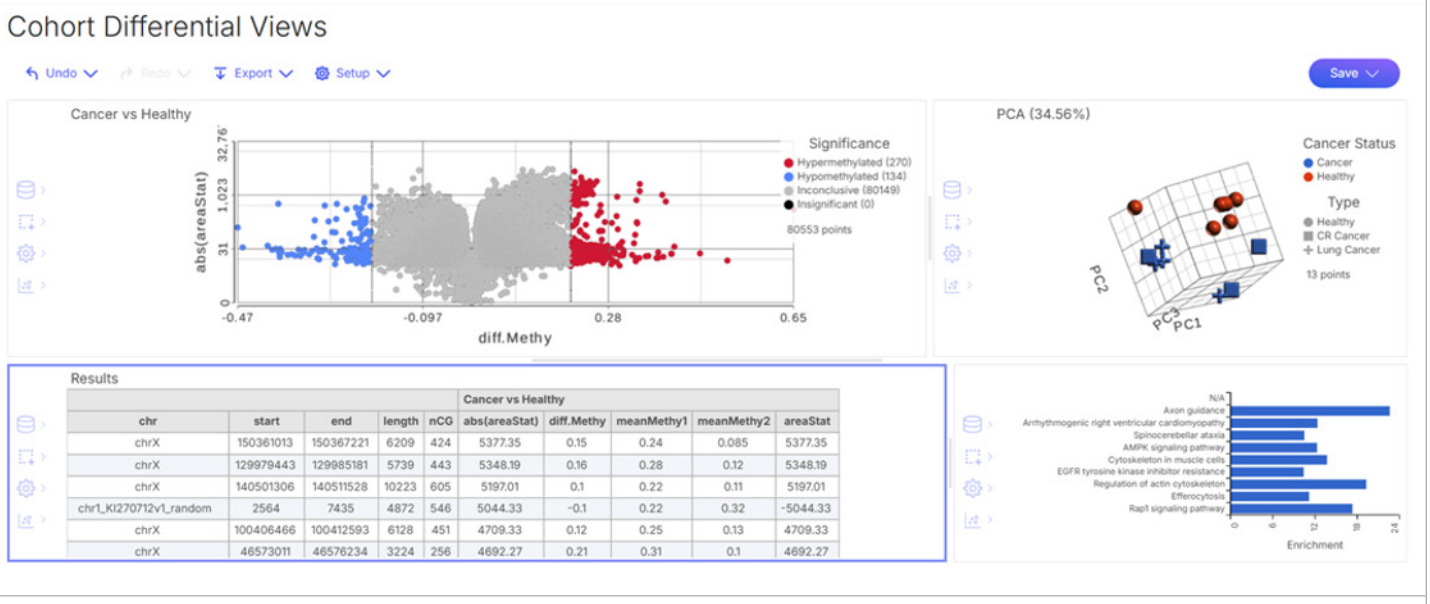


Figure 7: Interpret results with Illumina Connected Multiomics

Run default pipelines and build custom workflows for exploration through discovery using Illumina Connected Multiomics. Versatile dashboard visualizations provide deep insights with options to annotate genomic regions, identify differentially methylated regions, explore biological groupings with unsupervised clustering, and capture pathways associated with methylation changes.

High-quality dual insights

Sequencing performance from Illumina 5-Base DNA Prep shows high coverage of CpGs and uniform coverage across a range of GC content in the human genome (Figure 8). Illumina 5-Base DNA Prep libraries also show high library yield with a minimal number of PCR cycles and exceptional mapping efficiency (Figure 9). The measured methylation levels for each sample are reproducible across different inputs (Figure 10) and technical replicates (Figure 11). 5mC conversion is highly selective across a range of sample inputs, as measured by small genome control spike-ins (Figure 12). Highly accurate germline variant calling for SNVs, insertions-deletions (indels), and copy number variants (CNVs) enables the comprehensive insights of parallel WGS and methylation assays in a single workflow (Figure 13). Detection of 5mC and genetic bases from the same molecules allows for allele-specific resolution of methylation events and genetic variants for phased data (Figure 14).

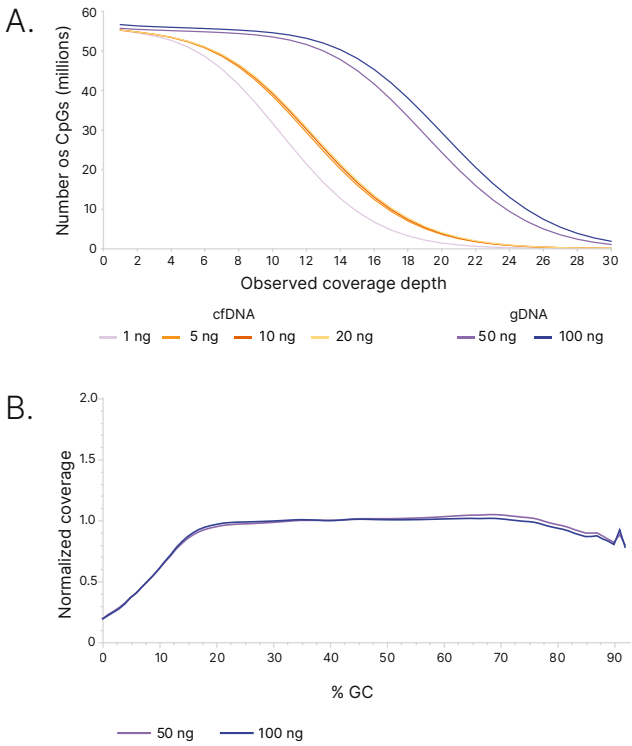


Figure 8: Exceptional sequencing performance with Illumina 5-Base DNA Prep

(A) High CpG coverage across 1–20 ng cfDNA and 50–100 ng gDNA inputs. (B) Even GC coverage across 50–100 ng gDNA inputs. cfDNA is extracted from serum of healthy donors; gDNA is reference human genome sample NA12878 (Coriell Institute for Medical Research). Libraries were sequenced on the NovaSeq X System to 500M clusters.

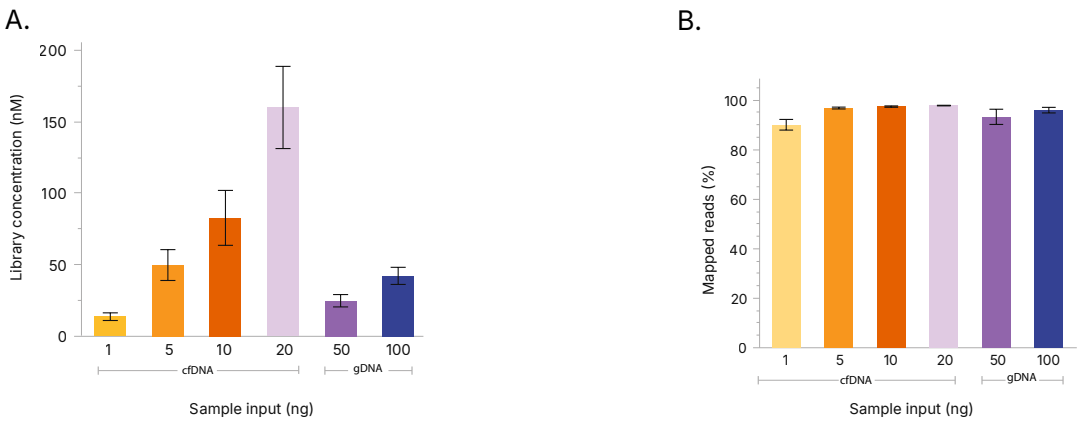


Figure 9: High yield and alignment rates using Illumina 5-Base DNA Prep

(A) Library yields and (B) alignment rates (percent mapped reads) using Illumina 5-Base DNA Prep for 1–20 ng inputs of cfDNA and 50–100 ng inputs of gDNA. cfDNA is extracted from serum of healthy donors; gDNA is reference human genome sample NA12878. Libraries were sequenced on the NovaSeq X System.

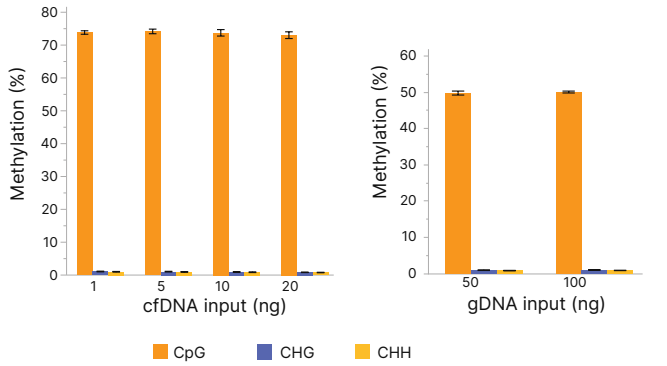


Figure 10: Methylation detection with Illumina 5-Base DNA Prep across a range of sample types and DNA inputs

Percent methylation of cytosines in CpG, CHG, and CHH context. cfDNA extracted from serum of healthy donors, where global CpG methylation of 70–80% is expected. Very low levels of CHG and CHH methylation are observed as expected. Cell line–derived gDNA from reference human genome sample NA12878, where global CpG methylation of ~50% is expected. Libraries were sequenced on the NovaSeq X System. Secondary data analysis was performed with DRAGEN Germline v4.4.4.

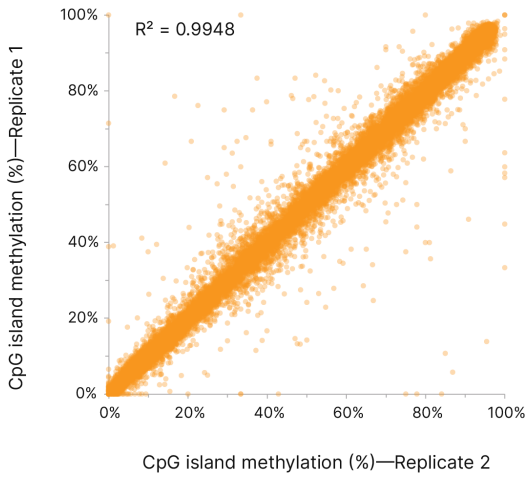
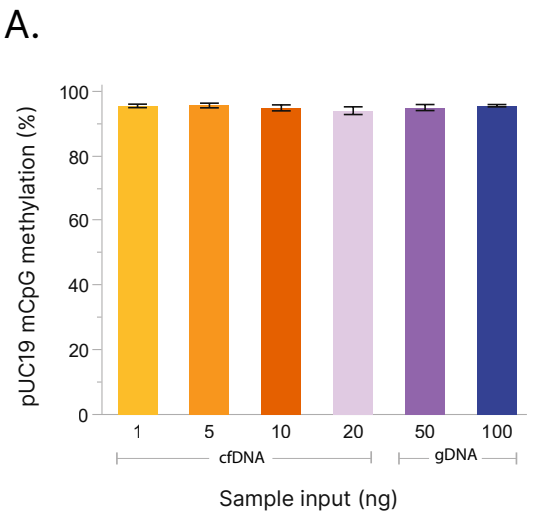


Figure 11: Reproducible methylation measurement at CpG islands across all methylation levels with Illumina 5-Base DNA Prep

Correlation of mean methylation level between two replicates of reference human genome sample NA12878 for all CpG island regions. Libraries were sequenced on the NovaSeq X System. Secondary data analysis was performed with DRAGEN Germline v4.4.4.



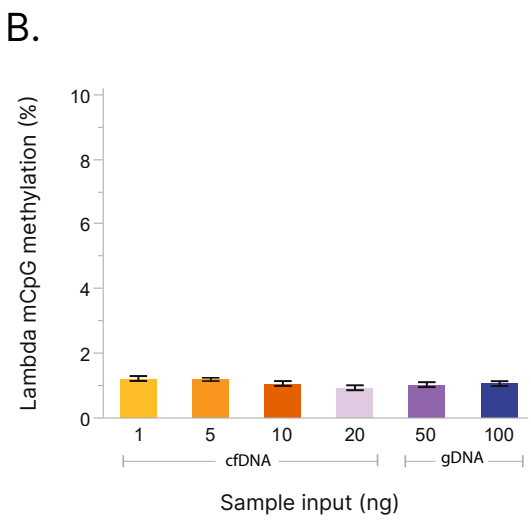


Figure 12: Selective methylation conversion with Illumina 5-Base DNA Prep

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 QC. Input amounts are 1–20 ng cfDNA from healthy donors and 50–100 ng gDNA from human reference sample NA12878.

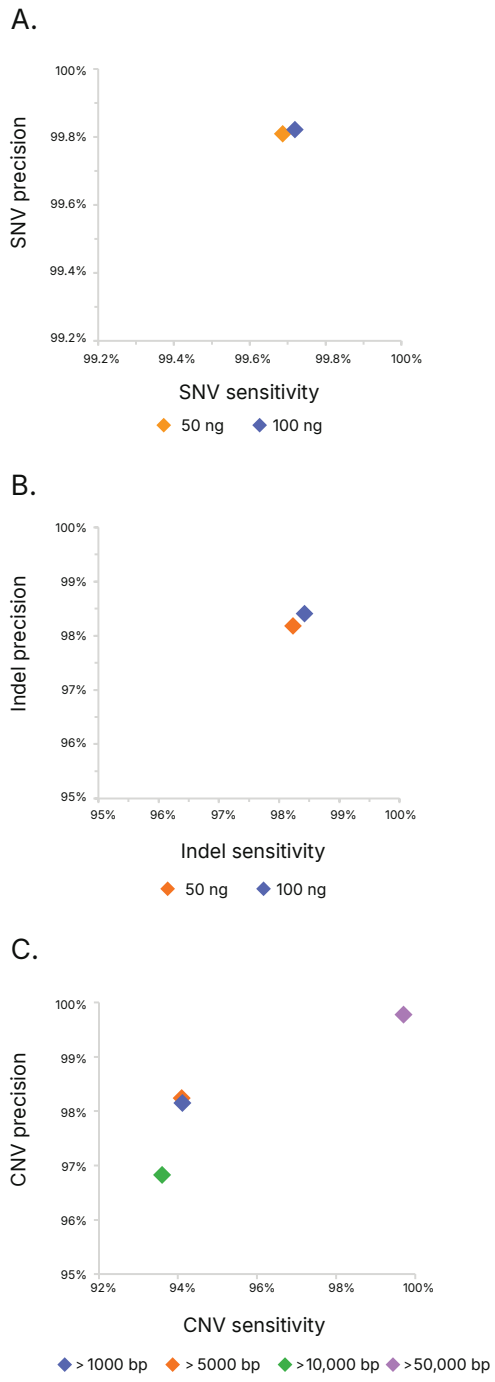


Figure 13: High-accuracy germline variant calling with Illumina 5-Base DNA Prep

(A) SNV and (B) indel variant calling for 50 ng and 100 ng input gDNA prepared from reference human genome sample NA12878. (C) Germline CNV deletion accuracy separated by variant size with reference human genome sample HG002 (Genome in a Bottle). Libraries were sequenced on the NovaSeq X System and subsampled to 500M reads.

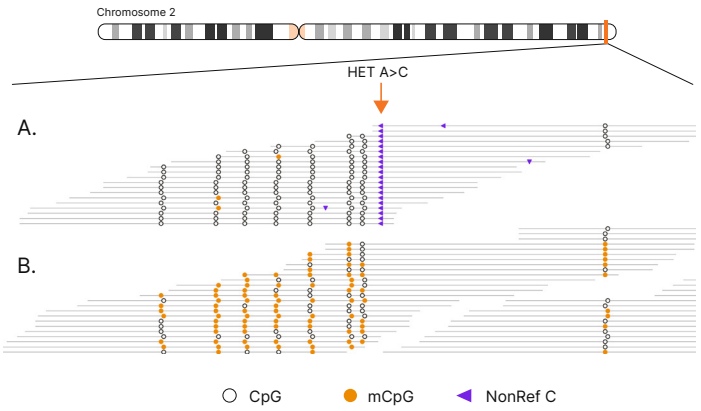


Figure 14: Resolution of both genetic variants and methylation on the same allele

Illumina 5-Base DNA Prep reveals allele-specific methylation patterns. DRAGEN Germline pipeline with 5-base methylation analysis can align both genetic variant and methylation data from the same reads. Detailed view showing an intron of the *RAMP1* gene on chromosome 2. (A) unmethylated allele and (B) methylated allele. Libraries were prepared from reference human genome sample NA12878.

Cost-effective whole-genome discovery

Illumina 5-base DNA Prep offers the lowest total workflow cost relative to alternative NGS methods.[6] For methylation-focused applications, the Illumina 5-base solution requires less sequencing than standard methylation assays due to greater mapping efficiency and CpG coverage per run. For dual insights, Illumina 5-Base DNA Prep delivers methylation profiling and genomic variant calling with minimal incremental sequencing cost relative to standard WGS alone.‡ Highly efficient dual-omic DRAGEN secondary analysis also reduces NGS data analysis costs.

‡ Based on 500M read pairs (1B paired-end reads) for Illumina 5-Base DNA Prep vs 400M read pairs for standard WGS.

Summary

Combining analysis of genetic variation and DNA methylation helps maximize insights from every sample. Illumina 5-Base DNA Prep offers a library-to-interpretation solution for simultaneous genome and methylome profiling in one optimized and integrated workflow. Novel chemistry and algorithms deliver single-base resolution and high accuracy, while minimizing the amount of sequencing required. Comprehensive dual-omic reports using integrated DRAGEN analysis and Illumina Connected Multiomics help to accelerate biological discovery.

Learn more →

Illumina 5-Base DNA Prep

Ordering information

Product	Catalog no.
Library prep	
Illumina 5-Base DNA Prep (24 samples)	20140364
Illumina 5-Base DNA Prep (96 samples)	Coming soon
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
Illumina Connected Multiomics	Request a demo

References

1. Füllgrabe J, Gosal WS, Creed P, et al. Simultaneous sequencing of genetic and epigenetic bases in DNA. *Nat Biotechnol*. 2023;41(10):1457-1464. doi:10.1038/s41587-022-01652-0
2. Vaisvila R, Ponnaluri VKC, Sun Z, et al. Enzymatic methyl sequencing detects DNA methylation at single-base resolution from picograms of DNA. *Genome Res*. 2021;31(7):1280-1289. doi:10.1101/gr.266551.120
3. Babraham Bioinformatics. Bismark Bisulfite Mapper User Guide v0.15.0. bioinformatics.babraham.ac.uk/projects/bismark/Bismark_User_Guide.pdf. Published January 16, 2016. Accessed August 12, 2025.
4. Integrated DNA Technologies. xGen Methyl-Seq DNA Library Prep Kit protocol. sfvideo.blob.core.windows.net/sitefinity/docs/default-source/protocol/xgen-methyl-seq-dna-library-prep-kit-protocol.pdf?sfvrsn=9fa7e007_11. Published June 2023. Accessed August 12, 2025.
5. Illumina. Illumina DNA Prep data sheet. illumina.com/content/dam/illumina/gcs/assembled-assets/marketing-literature/illumina-dna-prep-data-sheet-m-gl-01373/illumina-dna-prep-data-sheet-m-gl-01373.pdf. Published 2023. Accessed August 12, 2025.
6. Biomodal. Duet multiomics solution +modC. biomodal.com/products/duet-modc/. Accessed August 12, 2025.

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