

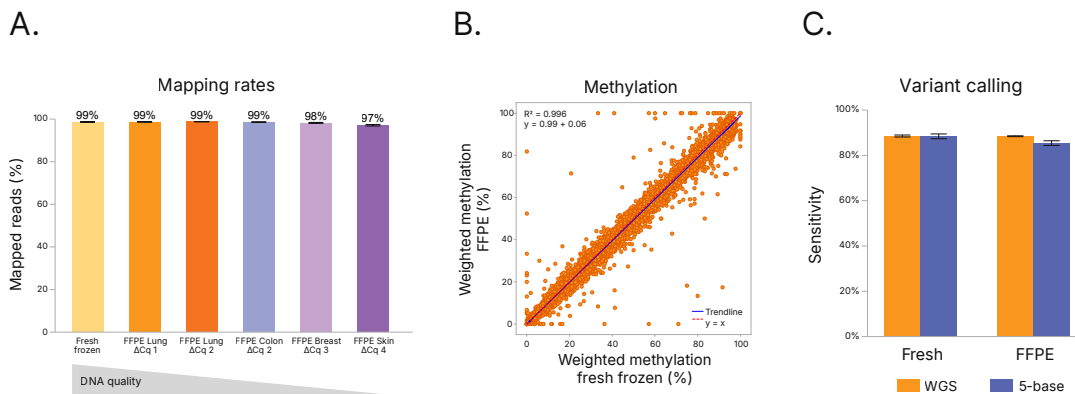
A single assay to simultaneously detect somatic variants and methylation signatures in FFPE samples

Illumina 5-Base DNA Prep

Formalin-fixed paraffin-embedded (FFPE) tissue samples are a valuable source of genomic material for cancer research. However, comprehensive molecular profiling of these samples is challenging due to the degraded quality of FFPE DNA.

Illumina 5-Base DNA Prep combines whole-genome sequencing (WGS) and methylation sequencing into one streamlined assay. The gentle single-step methylated base conversion preserves DNA integrity to maximize discovery power from FFPE samples. With Illumina 5-Base DNA Prep, researchers can obtain high-accuracy methylome insights and somatic variant calling.

Illumina 5-Base DNA Prep is compatible with FFPE samples

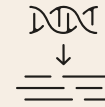


(A) Genomic DNA (gDNA) from a fresh frozen cell line and FFPE DNA from lung, colon, skin, and breast cancer tissue were prepared using Illumina 5-Base DNA Prep. All sample types generated high-complexity libraries with mapping rates exceeding 97%. (B) A fresh frozen cancer cell line was formalin-fixed and paraffin-embedded to mimic FFPE processing. Methyl measurements and (C) single nucleotide variant (SNV) detection are concordant between fresh frozen and FFPE, with similar performance to WGS without methylation conversion. Samples sequenced on the NovaSeq X Series at ~37x coverage (500M clusters).



Prepare samples

Extract
FFPE DNA



Prepare libraries

Illumina 5-Base
DNA Prep



Sequence

NovaSeq™ X
Series



Analyze data

DRAGEN™
secondary analysis

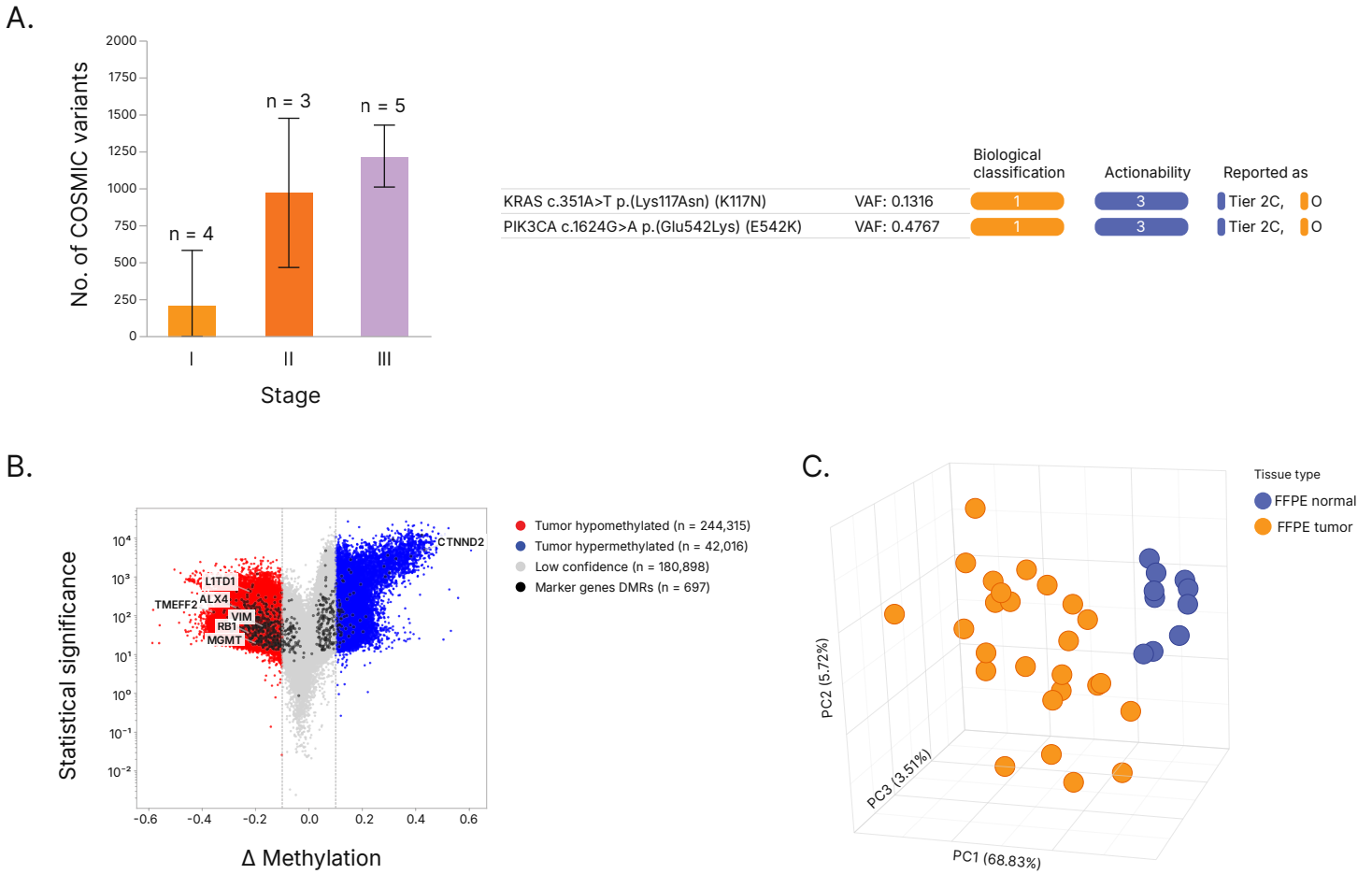


Interpret results

Illumina Connected
Multiomics

Illumina 5-Base DNA Prep detects genomic and epigenomic signatures of colorectal cancer (CRC) from FFPE tissue

DNA from FFPE samples of CRC tumors and adjacent normal tissue was prepared with Illumina 5-Base DNA Prep and analyzed using 5-base-aware DRAGEN algorithms to generate simultaneous variant calling and methylation results. Tumor-normal analysis identified cancer-associated variants specific to each tumor. Illumina Connected Multiomics analyzed genome-wide methylation profiles to identify differentially methylated regions (DMR) between tumor vs paired normal FFPE samples and perform principal component analysis (PCA).



(A) Variant interpretation performed with Illumina Connected Insights found tumor-specific small variants that overlap with variants in the COSMIC* data base. Error bars are the standard deviation across different tumor samples, binned by cancer stage. Screenshot on the right shows two example detected small variants classified as oncogenic in a tumor sample. (B) Volcano plot of DMRs detected with tumor-normal analysis shows accurate identification of both hypomethylated DMRs (red) and hypermethylated DMRs (blue). (C) PCA clustering of samples by methylation profiles for CRC FFPE samples plus matched adjacent normal FFPE samples. All samples sequenced on the NovaSeq X Series at 150x–200x coverage. Secondary analysis used DRAGEN Somatic pipeline v4.4.6.

* COSMIC, Catalogue of Somatic Mutations in Cancer.

See our recommended protocol for using Illumina 5-Base DNA Prep with FFPE samples



Learn more about Illumina 5-Base DNA Prep →

www.illumina.com/5-base