Illumina 5-base sequencing resolves complex central nervous system cancers and rare and undiagnosed genetic diseases

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Introduction

- Multiomic approaches provide predictive insights into the phenotypes of biological systems. For example, in glioblastoma profiling, both SNVs in *EGFR* and *MGMT* promoter methylation are prognostic biomarkers that inform treatment decisions. In genetic disease, imprinting disorders require both genome and DNA methylation analyses to identify pathogenic changes. However, multiomic approaches are often time-consuming, and require considerable material input, presenting a significant barrier to widespread adoption.
- Illumina 5-base DNA Prep a novel single-step methyl-conversion method and DRAGEN analysis that enables detection of both genomic variants and DNA methylation from a single workflow
- We paired Illumina 5-base Prep paired EpiSign classifiers and Emedgene variant analysis to evaluate 3 RUGD disease case previously resolved through separate methylation and WGS assays.
- Additionally, we combined Illumina 5-base Prep with tumor classification algorithms powered by Epignostix CNS tumor classifier to assess central nervous system (CNS) tumor samples that have previously been classified through methylation array.
- We demonstrate that Illumina 5-base Prep with DRAGEN analysis and EpiSign and Epignostix classifiers resolve multiomic RUGD and CNS cases with a single workflow.

Introducing the Illumina 5-base solution

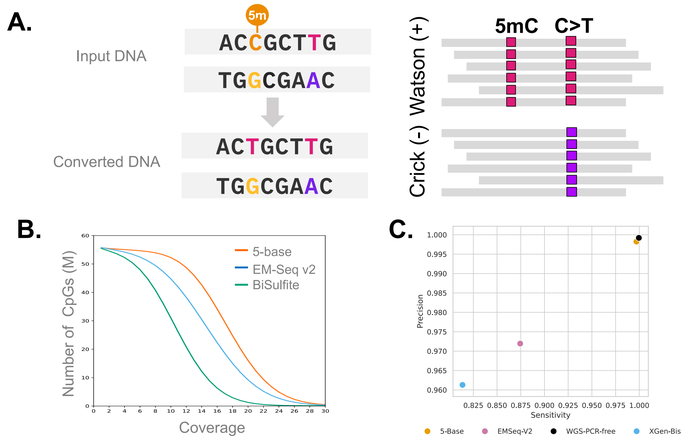

Figure 1: Introducing Illumina 5-base (A) Illumina 5-Base DNA Prep leverages unique chemistry and optimized DRAGEN analysis algorithms to enable simultaneous methylation and variant calling from a single sample. (B) 5-base has more comprehensive coverage of CpG sites compared to EM-Seq v2 and Bisulfite at equivalent reads (C) Illumina 5-base enables high accuracy germline SNV calling approaching WGS performance. EM-Seq and Bisulfite variant calling was processed by Bis-SNP, 5-base and WGS by DRAGEN.

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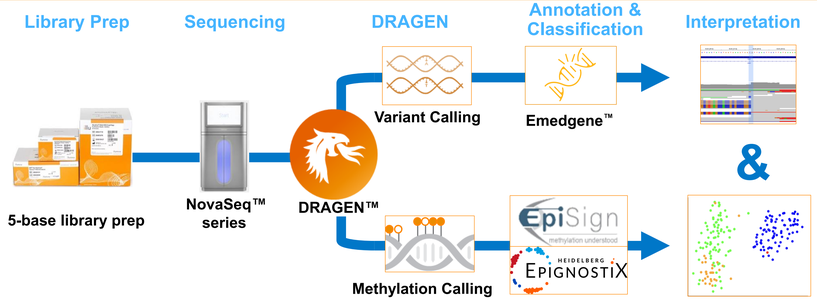
5-base integrates with classification & interpretation tools



Illumina 5-base analysis output is compatible with a wide range of tertiary analysis tools including Illumina Connected Multiomics, Emedgene, and methylation classifiers developed by Epignostix and EpiSign.

5-base application with Epignostix for CNS classification

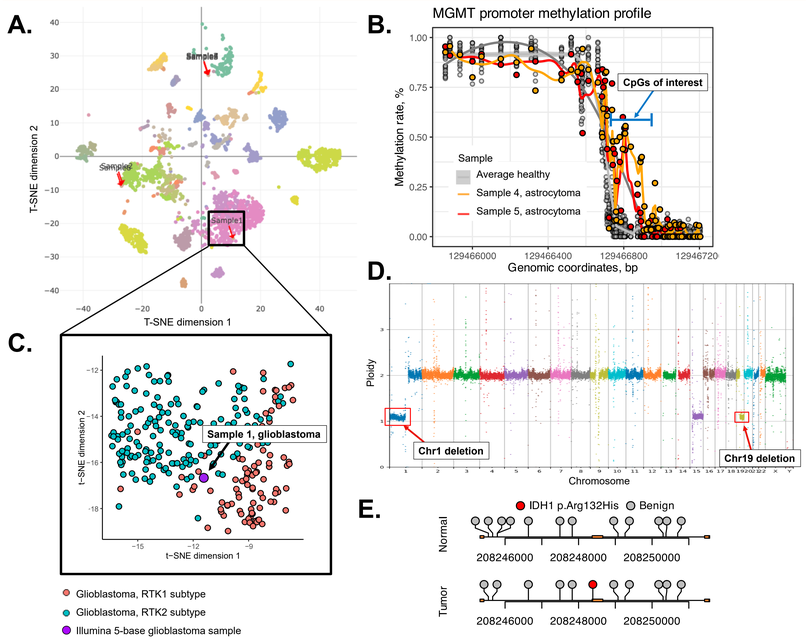


Figure 2: Illumina 5-base method applied to CNS classification. (A) Epignostix previously developed a robust machine learning model for classification of CNS tumors using DNA-methylation signatures from 7,495 samples across 184 tumor classes processed with Illumina Infinium Methylation arrays.[1] In addition to classification, the method evaluated MGMT gene promoter methylation status. We performed Illumina 5-base DNA Prep and DRAGEN analysis plus Epignostix classification for 8 CNS tumor samples previously analyzed by array. (B) We observe concordant MGMT gene promotor methylation status compared to the healthy samples cohort (C), and classification of the glioblastoma methylation signatures. (D) The Illumina 5-base methylation signal additionally detects the deletions on chr1 and chr19 defining the tumor subclass. (E) In addition to methylation signals, we identify an *IDH1* Arg132His mutation, a marker relevant for patients' management for gliomas.

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5-base application to EpiSign for RUGD cases resolution

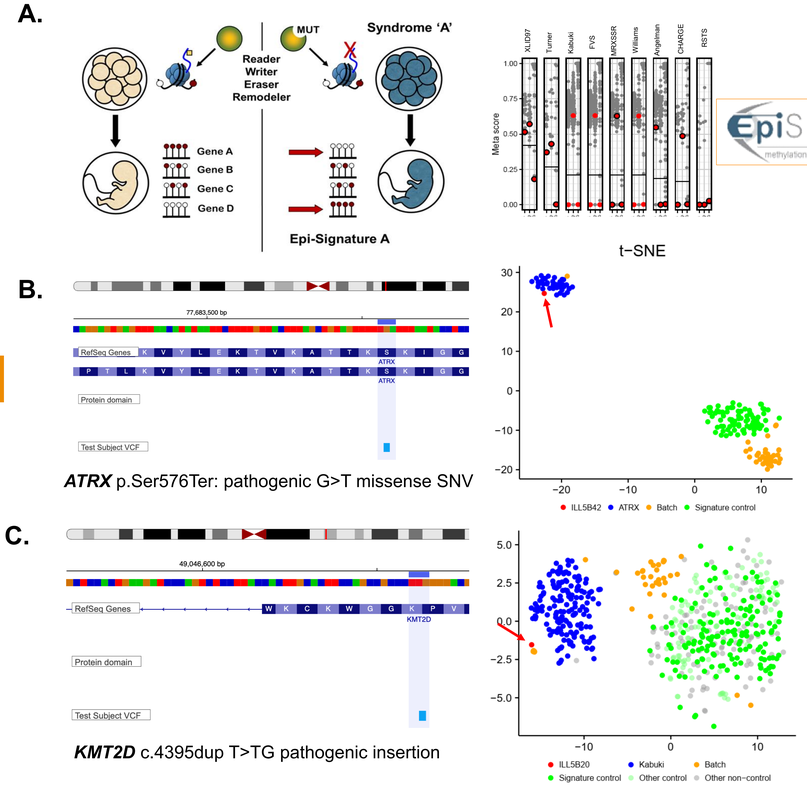

Figure 3: Illumina 5-base resolves complex multiomic RUGD cases. EpiSign has developed bespoke pipelines for classification of RUGD cases using Illumina Infinium methylation arrays.[2] We performed Illumina 5-base DNA Prep and DRAGEN analysis on a cohort of 48 RUGD samples with disease episignatures. We resolved all 48 cases, and here we show 2 RUGD samples from this cohort that were not previously resolved by WGS alone. (B) We classified the first case as alpha-thalassemia intellectual disability syndrome by detecting a de novo pathogenic missense variant in the *ATRX* gene consistent with pathogenic methylation and by EpiSign classification based on methylation signation (C). In the second case, Kabuki syndrome classification was assigned from a *KMT2D* pathogenic insertion and EpiSign classification of the disease episignatures.

Summary

Illumina 5-base DNA Prep and DRAGEN analysis paired with powerful methylation classifiers show great potential for RUGD and CNS classification using a highly streamlined, scalable dual-omic workflow.

[1] Capper D, Jones DTW, Sill M, et al. DNA methylation-based classification of central nervous system tumours. Nature. 2018;555(7697):469-474. doi:10.1038/nature26000

[2] Sadikovic, B., Levy, M.A., Kerkhof, J. et al. Clinical epigenomics: genome-wide DNA methylation analysis for the diagnosis of Mendelian disorders. Genet Med 23, 1065–1074 (2021). https://doi.org/10.1038/s41436-020-01096-4

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