

CNS Tumor Methylation Classification using duet evoC

- [MNP-Flex v1.1.0](#) accepts whole-genome sequencing (WGS) DNA methylation data prepared using biomodal's duet evoC, from both fresh frozen (FF) and formalin-fixed, paraffin-embedded (FFPE) specimens,
- [MNP-Flex Processing](#) performs automatic standardization of methylation data for seamless downstream classification of CNS tumors,
- A fully offline, static [MNP-Flex Classifier](#) delivers reproducible and secure analysis, with no network access required at runtime,
- [MNP-Flex](#) with duet evoC data delivers 100% classification concordance with array at every hierarchical level — superfamily, family, class, and subclass.

Overview

MNP-Flex is our platform-agnostic CNS tumor methylation classifier developed for WGS data¹. **MNP-Flex** can predict 184 CNS tumors defined by the array-based Heidelberg Epignostix CNS Tumor Methylation Classifier v12.8², across a hierarchical structure with >99% accuracy. To extend the classifier beyond array data to emerging sequencing chemistries, we developed **MNP-Flex Processing**, which ingests input files of heterogeneous formats and harmonizes them onto the representation the model was trained on. **MNP-Flex Classifier** uses a static model based on a gradient-boosted tree ensemble trained on binarized beta-methylation values. **MNP-Flex Classifier** and **MNP-Flex Processing** are distributed together as a single offline Docker container, so that a given input returns an identical result on any compliant host — a prerequisite for reproducible and auditable deployment.

biomodal's duet evoC enables simultaneous methylation profiling and high-accuracy genetic variant calling from a single library³. The chemistry is enzymatic rather than bisulfite-based, preserves high-quality genetics and delivers highly sensitive and specific 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) calls. Through duet's proprietary hairpin and read resolution approach, sequence complexity and alignment quality are retained, and each read is resolved to the 6-base alphabet — A, G, C, T, 5mC, and 5hmC — at single-base resolution.

This Technical Note describes the application of **MNP-Flex** to data generated with biomodal's duet evoC, a combination that delivers accurate CNS tumor classification together with copy number variation (CNV) and mutation calling — from one sample, in a single workflow and from either FF- or FFPE-derived DNA. This consolidates methylation-based classification and molecular characterization, which typically require separate assays, into a single workflow.

Key points

- Simultaneous methylation profiling with concurrent mutation and CNV calling from a single sample using either FF or FFPE tissues,
- Classification compatible with the Heidelberg Epignostix CNS Tumor Methylation Classifier v12.8 across 184 subclasses,

- 100% concordance with the reference classification at all hierarchical levels across all FF and FFPE specimens tested,
- Fully offline, containerized (Docker) deployment — no network access required at runtime — for reproducible, regulated analysis.

Workflow Summary

The end-to-end workflow proceeds from FFPE or FF tissue through DNA extraction, duet evoC library preparation, sequencing, secondary analysis using the duet software, and offline classification with [MNP-Flex](#) ([Fig. 1](#)). Every step downstream of sequencing is automated, and the final classification step executes without network access.

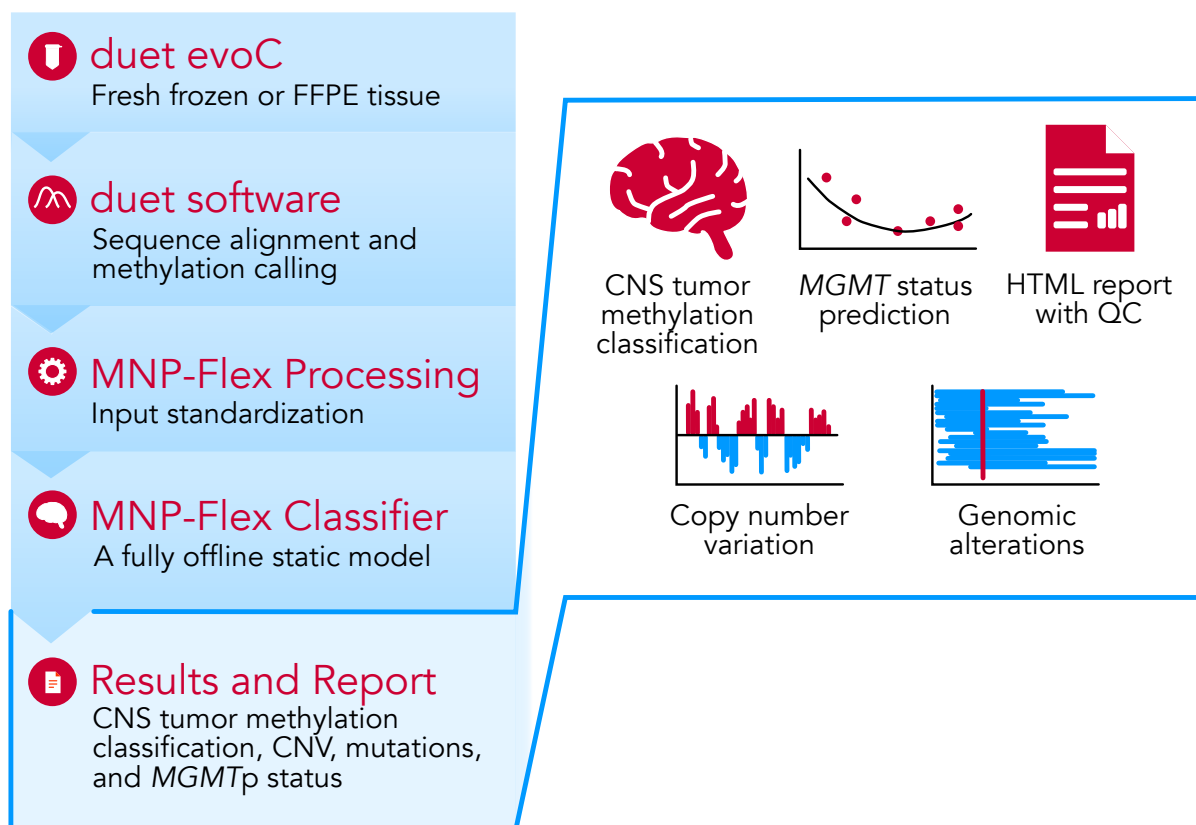


Figure 1. CNS tumor methylation classification workflow overview. Tissue (FF or FFPE) is processed through DNA extraction and duet evoC library preparation, sequenced on a standard short-read platform, and analyzed with the duet software to yield methylation calls together with mutation and CNV data. Methylation output is standardized by [MNP-Flex Processing](#) and classified offline by [MNP-Flex Classifier](#), returning a score and a subclass, class, family, and superfamily assignment.

biomodal's duet evoC

biomodal's duet evoC enables highly accurate 6-base WGS and methylation analysis from a single sample³. The protocol is compatible with DNA from FF or FFPE tissue and with any standard short-read sequencing platform, so it can be adopted without changes to existing sequencing infrastructure. Because methylation and genetic information derive from the same reads, methylation calls and variant calls are intrinsically matched.

biomodal's duet software

biomodal's software resolves raw sequencing reads through to multiomic features, providing quality control, differential methylation analysis, and molecular alterations⁴. Its methylation output is consumed directly by [MNP-Flex](#), so no scripting is required between secondary analysis and classification.

Internal testing confirms that duet evoC yields high-quality methylation data from both FF- and FFPE-derived DNA ([Fig. 2](#)). duet generates 5mC, 5hmC, and 5modC values. The latter incorporates all cytosine modifications, mimicking the methylation array. As a result, quantile–quantile comparison of 5modC against matched array data follows the diagonal closely across the full methylation range for both materials, with only modest deviations at intermediate methylation values. The underlying distributions are strongly bimodal, as expected for genome-wide CpG methylation. FFPE libraries are therefore not the limiting material: archival tissue is as informative as FF tissue for downstream classification.

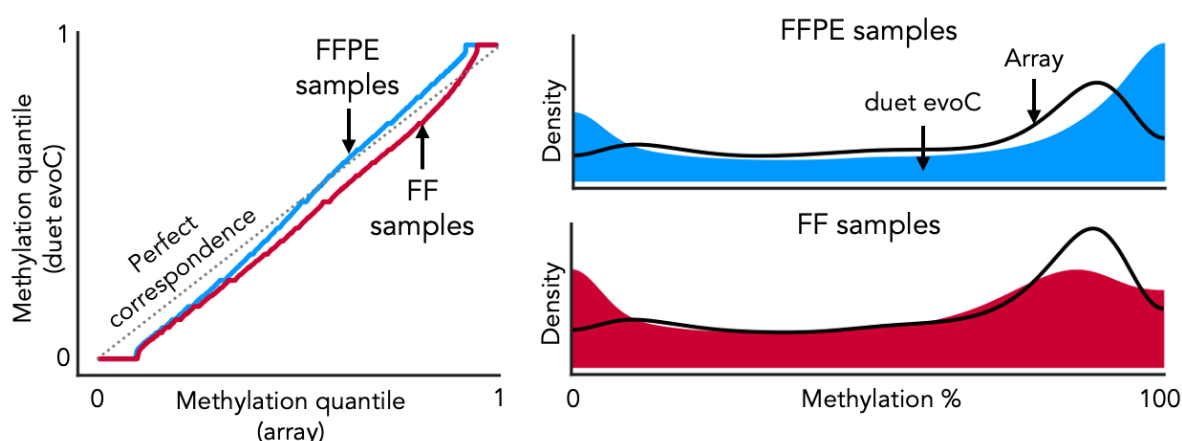


Figure 2. biomodal's duet evoC produces high-quality data from both FF and FFPE tissues. Left — quantile–quantile plot of duet evoC methylation quantiles against matched array quantiles for FFPE (blue) and FF (red) samples; the dotted line marks perfect correspondence. Right — density of methylation values for duet evoC (filled) overlaid on array data (black line), shown separately for FFPE and FF samples.

Preliminary analyses indicate that 5hmC is enriched ~1.7-fold at CpG sites relevant for tumor classification relative to the genome, suggesting a putative role for 5hmC in shaping the epigenetic patterns that distinguish CNS tumor entities.

MNP-Flex Processing

MNP-Flex Processing sits between secondary analysis and classification and standardizes the input. Methylation calls arriving in different file formats, coordinate conventions, and value scales are mapped onto the single representation **MNP-Flex Classifier** expects, so that no manual reformatting is required for a new data source.

MNP-Flex Classification

MNP-Flex is a containerized, offline methylation classification platform developed by Heidelberg Epignostix. It accepts a single DNA methylation input file and returns a structured result set: hierarchical class assignments with their associated scores, quality-control metrics, diagnostic figures, and a human-readable summary report. Because the model is static, an input always yields the same output — there is no retraining, model drift, or version ambiguity between runs, and no internet connection is required at any point.

- **Input:** **MNP-Flex** accepts the CX report file generated by duet software from a single sample with modC values
- **Process run:** a Docker container with mounted input/output volumes; no network access is required at runtime, supporting reproducible deployment in regulated environments
- **Output:** **MNP-Flex** prediction and score for four levels (superfamily, family, class, subclass), predicted *MGMT*p status, mutations, CNV, and an HTML report with results summary and quality-control metrics.

Summary

Together, biomodal's duet evoC and **MNP-Flex** deliver CNS tumor methylation classification with concurrent mutation and CNV calling from a single library, from either FF- or FFPE-derived DNA. Methylation classification was concordant with the matched array predictions at all four hierarchical levels for every specimen tested, and FFPE material performed on a par with fresh

frozen tissue — removing the practical constraint that has limited methylation classification of archival specimens.

MNP-Flex Processing is what makes this possible: by standardizing input formats and correcting platform-specific shifts, it allows a model trained on array data to be applied unchanged to a sequencing-based chemistry. The same mechanism allows generalization to further platforms without retraining. Delivered as a single offline container with a static model, the workflow is reproducible by construction and suited to secure, regulated research environments.

Owing to the enrichment of 5hmC in brain tissue, and specifically at classification-relevant genomic regions, resolving 5hmC from 5mC may help unravel the epigenetic intricacies of CNS tumors.

Visit epignostix.com for more information or contact us at support@epignostix.com.

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References

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2. Sill, M., Schrimpf, D., Patel, A. *et al.* Advancing CNS tumor diagnostics with expanded DNA methylation-based classification. *Cancer Cell* **44**(2), 340-354 (2026). <https://doi.org/10.1016/j.ccell.2025.11.002>
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