

CNS Tumor Methylation Classification using Watchmaker TAPS+

- **MNP-Flex v1.1.0** accepts whole-genome sequencing (WGS) DNA methylation data prepared using the Watchmaker DNA Library Prep Kit with TAPS+, 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 Watchmaker's TAPS+ 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.

The Watchmaker DNA Library Prep Kit with TAPS+ is a positive-readout methylation sequencing chemistry that converts methylated cytosines (5mC and 5hmC) to thymines while preserving unmethylated cytosines³. This maintains base complexity (ATCG) and delivers 5mC and genomic alteration detection from a single library for multimodal analysis across discovery, translational, and population studies, as well as multi-cancer early detection and monitoring applications. Its nondamaging workflow delivers robust performance with degraded FFPE and low-input samples — down to 1 ng.

This Technical Note describes the application of **MNP-Flex** to data generated with the Watchmaker DNA Library Prep Kit with TAPS+, a combination that delivers accurate CNS tumor classification together with copy number (CNV), structural (SV), and single nucleotide (SNV) variant calling — from one library, and from either FF- or FFPE-derived DNA. This consolidates methylation-based classification and molecular characterization, which conventionally require separate assays, into a single workflow.

Key points

- Simultaneous methylation profiling with concurrent SNV, SV, and CNV calling from a single library 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, TAPS+ library preparation, short-read sequencing, secondary analysis to generate per-CpG methylation calls, and offline classification in [MNP-Flex](#) (Fig. 1). Every step downstream of sequencing is automated, and the final classification step executes without network access.

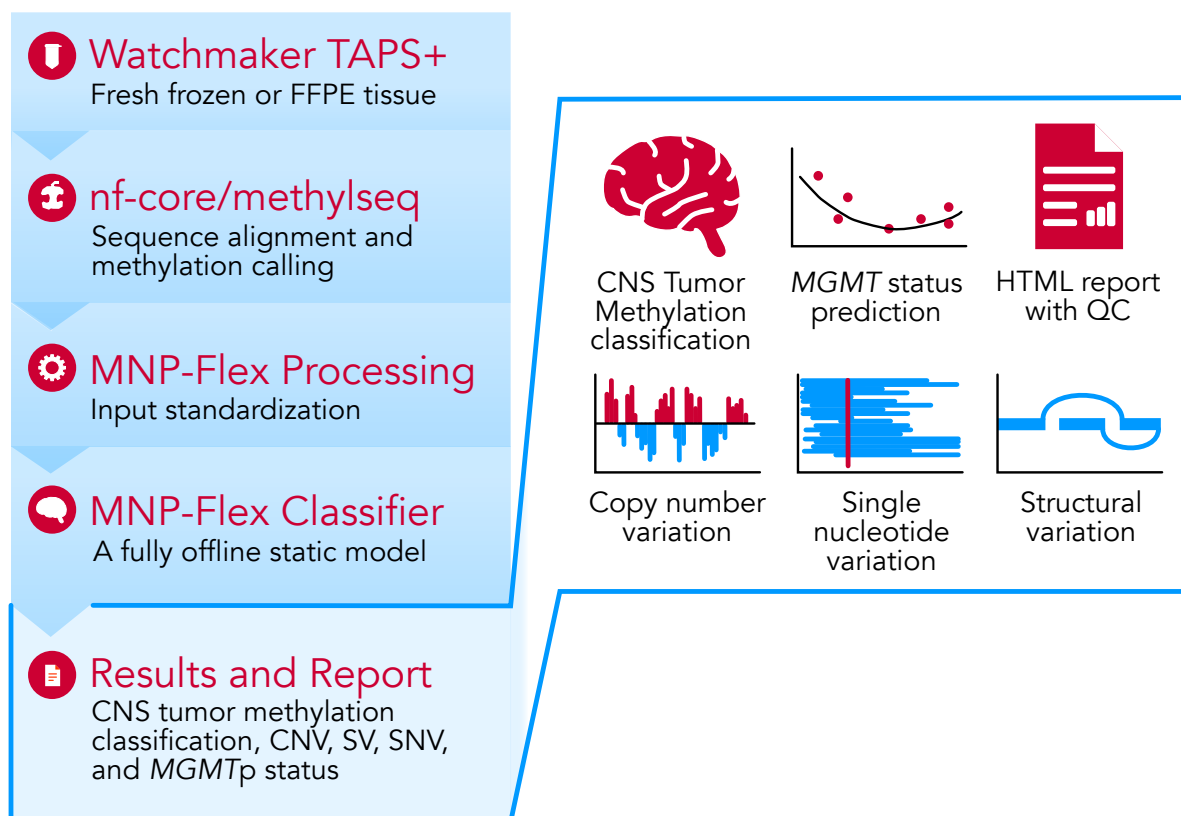


Figure 1. CNS tumor methylation classification workflow overview. Tissue (FF or FFPE) is processed through DNA extraction and library preparation with the Watchmaker DNA Library Prep Kit with TAPS+, sequenced on a standard short-read platform, and analyzed with the secondary analysis pipeline (nf-core/methylseq) to yield methylation calls together with SNV, SV, 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.

Watchmaker DNA Library Prep Kit with TAPS+

The Watchmaker DNA Library Prep Kit with TAPS+ enables highly accurate 5-base WGS and methylation analysis from a single library³. 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.

TAPS+ secondary analysis

Several software tools are available for analyzing TAPS+ data⁴. The recommended open-source workflow — nf-core/methylseq⁵ — provides a flexible and transparent approach for alignment, methylation calling, quality control, and variant detection. The methylation output is consumed directly by [MNP-Flex Processing](#), so no manual reformatting is required between secondary analysis and classification.

Internal testing confirms that Watchmaker TAPS+ yields high-quality methylation data from both FF- and FFPE-derived DNA ([Fig. 2](#)). Because TAPS+ converts both 5mC and 5hmC, its calls report total cytosine modification — the same quantity the methylation array measures. The data distributions are strongly bimodal for both materials, as expected for genome-wide CpG methylation. FFPE libraries are therefore not the limiting factor: archival tissue is as informative as FF tissue for downstream classification.

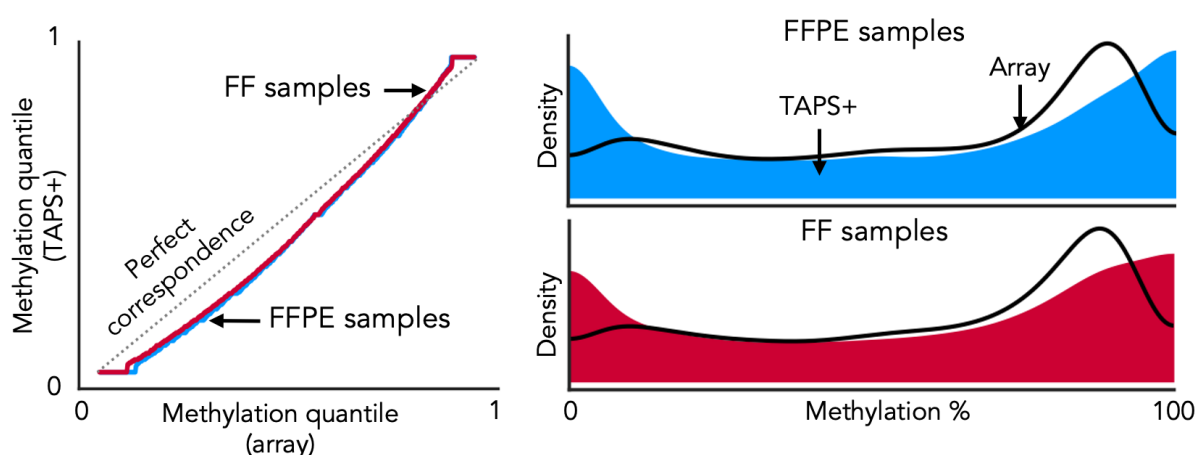


Figure 2. The Watchmaker DNA Library Prep Kit with TAPS+ produces high-quality data from both FF and FFPE tissues. *Left* — quantile–quantile plot of TAPS+ methylation quantiles against matched array quantiles for FFPE (blue) and FF (red) samples; the dotted line marks perfect correspondence. *Right* — density of methylation values for TAPS+ (filled) overlaid on array data (black line), shown separately for FFPE and FF samples.

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 methylation calls generated from a single TAPS+ library,
- **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, SNV, SV, CNV, and an HTML report with results summary and quality-control metrics.

Summary

Together, the Watchmaker DNA Library Prep Kit with TAPS+ and **MNP-Flex** deliver CNS tumor methylation classification with concurrent SNV, SV, 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 generalizes 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.

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

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References

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3. Watchmaker Genomics. Watchmaker DNA Library Prep Kit with TAPS+. *Watchmaker Genomics*, Boulder, CO, USA (2025). https://www.watchmakergenomics.com/media/wg/asset/%2Fm%2F4%2Fm422_taps_ss_wm113_v1-0-1125.pdf
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