

JAX OncoMethyl Array® – CNS Tumor

Patient

Name: John Doe
 Patient ID: ABC123
 Source Patient ID: 0123456
 Date of Birth: 01/01/1960
 Sex: Male
 Submitted Diagnosis: Infiltrating glioma

Specimen

Specimen ID: S9876543
 Source Specimen ID: X26-01234
 Primary Tumor Site: Brain
 Specimen Site: Left frontal lobe
 Neoplastic Content: 75%
 Collection Date: 03/25/2026
 Received Date: 04/01/2026

Physician

Name: Dr. Gene
 Affiliation: Genomics Institute

Methylation profiling is intended to provide supplemental information for the diagnosis of central nervous system tumors. The final diagnosis must be made by a qualified physician in the context of the patient's clinical history and test results.

CNS Tumor Methylation Profiling Results^{1,2}

Adult-type diffuse gliomas

*Super Family Calibrated Score: 0.9999

Diffuse glioma, IDH-mutant

*Family Calibrated Score: 0.9998

Diffuse glioma, IDH-mutant and 1p19q-retained (astroglial type)

*Class Calibrated Score: 0.9988

Astrocytoma, IDH-Mutant, lower grade

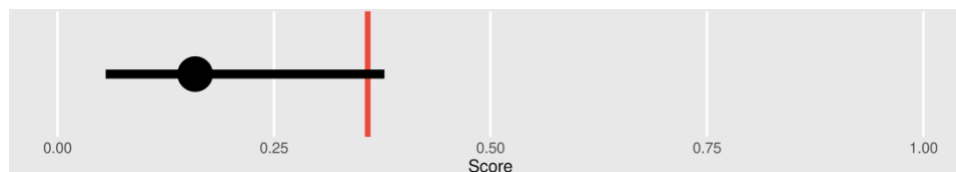
*Subclass Calibrated Score: 0.9988

Evidence Level†: B

Description:

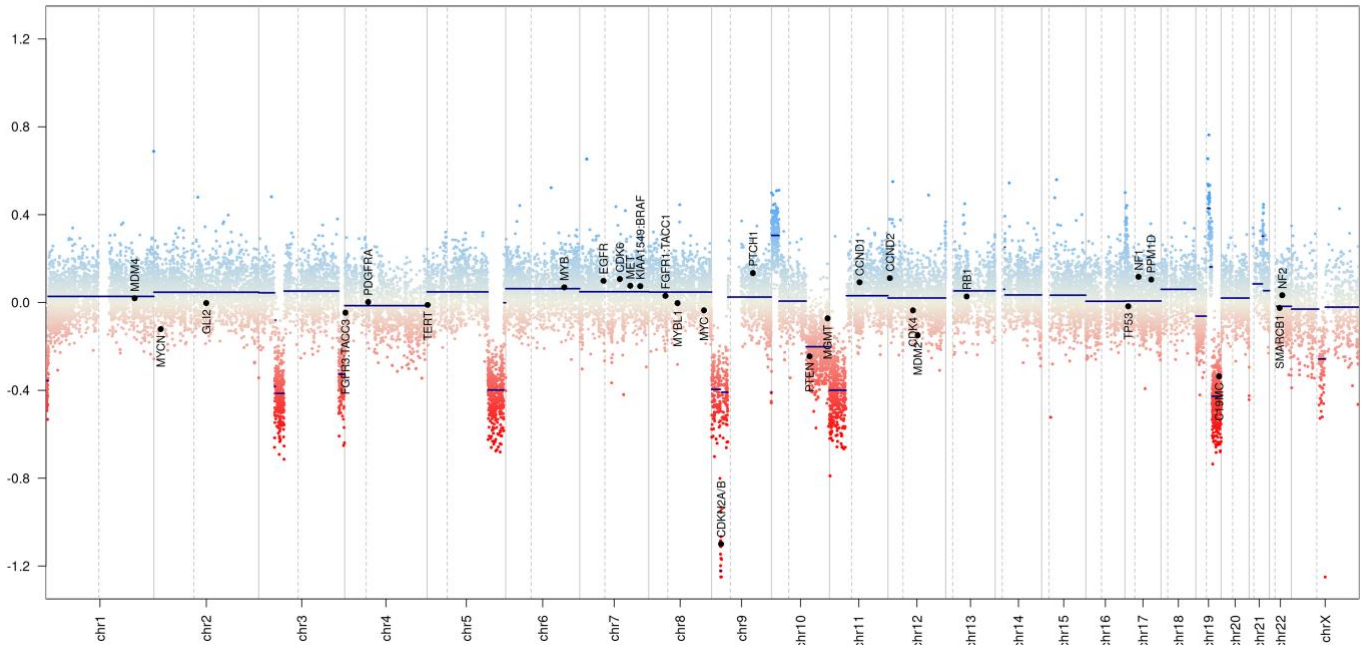
The "astrocytoma, IDH-mutant, lower-grade" subclass mainly comprises tumors with astrocytic histology of CNS WHO grades 2 and 3. This class universally harbors mutations of either IDH1 or IDH2 and the associated glioma CIMP phenotype. Tumors of this class usually have a supratentorial location but may also arise infratentorially; median age is 35 years (age range 16 to 71 years). Complete 1p/19q codeletion is not compatible with "astrocytoma, IDH-mutant" and if present should lead to diagnosis of an "oligodendroglioma, IDH-mutant and 1p/19q-codeleted" despite a possibly higher classifier score for astrocytoma.

MGMT Promoter Methylation Status³: Indeterminate



Copy Number Variation[‡]:

Chromosomal Alterations	Gene Gains	Gene Losses
+6q, -9p, +10p, -10q, -11p, -19, +21q	None	<i>FGF1, MTAP, CDKN2A, CDKN2B</i>



Footnotes

*Calibrated scores represent an estimated likelihood measure of methylation class assignment. Pathologists should be wary about endorsing suggested diagnoses with scores below 0.84 and should discard recommendations if scores are below 0.50^{1,2}. The JAX OncoMethyl Array® – CNS Tumor has been validated for samples with methylation class calibrated scores ≥ 0.50 only.

[†]Evidence levels are defined as follows²: Level A - Tumor type/subtype identical to WHO 2021; Level B - Large, single or more than one smaller dataset published describing the type/subtype as molecularly and/or clinically distinct, or the methylation class represents a distinct fraction of an established WHO 2021 tumor class; Level C - Single small dataset or case series; Level D - Solely based on clusters in tSNE/UMAP.

[‡]Copy number profile depicts chromosomes 1-22 (and X/Y if automatic prediction was successful). Copy number gains/amplifications are shown as positive deviations, while copy number losses are shown as negative deviations from the baseline. Fifty-five relevant gene regions are highlighted for reference⁴.

Quality Metrics Summary[§]: Pass

Detection Fraction	Red-to-Green Ratio	Mean Beta Value	CNV Noise
0.9638 (pass)	1.2814 (pass)	0.6951 (pass)	0.4082 (pass)

[§]Detection Fraction, Red-to-Green Ratio, and Mean Beta Value are computed using SeSAMe⁵ (see Methods for details). Detection Fraction refers to the fraction of probes with successful detection (p -value < 0.05). Red-to-Green Ratio indicates the red-to-green channel intensity ratio, which measures dye bias. Mean beta values indicate the mean methylation level across all probes. CNV Noise is obtained from Conumee2 implementation within the epxCNS package^{2,4}. CNV Noise measures the baseline variability in copy number signal across the genome using bin-level log₂ intensity ratios following segmentation.

Test Methods & Limitations

As necessary (for FFPE blocks or unstained slides), specimens are sectioned and stained using Fisher Chemical Eosin Y and Richard-Allan Scientific™ Hematoxylin Stain (Modified Mayer). Slides are digitally scanned on the Leica Aperio CS2 Scanner for remote pathologist review of neoplastic content, tissue type, tumor area, and specimen quality (Remote Testing Site: LBH07). Areas of high tumor cellularity ($\geq 70\%$ neoplastic content, NPC) are macro-dissected for DNA extraction using the QIAamp DNA FFPE Tissue Kit (Qiagen) or AllPrep DNA/RNA FFPE Kit (Qiagen), followed by bisulfite conversion (Zymo Research). Converted DNA undergoes whole genome amplification and processing with the Infinium MethylationEPIC Array v2 (Illumina). Bead chips are scanned on an Illumina iScan instrument.

Raw intensity data (IDAT files) are processed using the SeSAMe package (v1.28.1)⁵ in R to assess array-level quality. Metrics of detection fraction (acceptance range: 0.86 – 1.04), red-to-green ratio (acceptance range: 0.91 – 1.68), and mean beta (acceptance range: 0.54 – 0.71) are reviewed, and samples with two or more metrics outside acceptance criteria (defined as the average \pm two standard deviations across >400 representative samples) are failed and classification data not reported. For passing samples, IDAT files are then processed through the Epignostix CNS tumor methylation classifier package (epxCNS v1.1.3)^{1,2}. Additional quality assessment is performed for CNV noise (acceptance range: 0.1348 – 0.6726, based on mean \pm three standard deviations across >100 representative samples) prior to downstream copy number analysis; high CNV noise may result in poor CNV calling.

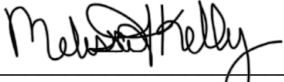
Methylation superfamily, family, class, and subclass predictions with calibrated probability scores and subclass description are provided by the classifier, which was trained on 7,495 methylation profiles spanning 184 tumor classes using a Random Forest approach with five-fold nested cross-validation.^{1,2} Methylation class calibrated scores

≥0.84 are considered “high confidence”, while scores <0.5 are deemed “no match”. Results with class calibrated scores between 0.5 and 0.84 are considered “suggestive” and should be interpreted with caution.

MGMT promoter methylation status is assessed using a logistic regression model (MGMT-STP27) comprising probes cg12434587 and cg12981137.³ Results are reported relative to the established probability cutoff of 0.3582, which was established based from samples with estimated average methylation of 7.28%. Samples are categorized as follows: “Methylated” when the estimated methylation probability and confidence intervals ≥0.3582; “Unmethylated” when the estimated methylation probability and confidence intervals <0.3582; or “Indeterminate” when the lower confidence interval <0.3582 and the upper confidence interval ≥0.3582.

Copy number variation (CNV) profile plots are generated from the methylation array intensity data using the Conumee2⁴ framework as implemented in the Epignostix epxCNS classifier package.² For CNV calling (gains and losses), probe-level log₂ intensity ratios are computed relative to an internal reference and segmented using circular binary segmentation. Chromosome-level (whole or arm) CNVs are reported for autosomes 1–22, excluding acrocentric arms 13p, 14p, 15p, 21p, and 22p. A Wilcoxon rank-sum test with rank-biserial correlation (RBC) as the effect size measure is applied. Variant calling thresholds for RBC are scaled by NPC to account for masking of CNVs in lower NPC samples (chr gain: RBC > 0.55×NPC and adjusted p < 0.01; chr loss: RBC < -0.55×NPC and adjusted p < 0.01). Gene-level CNVs are reported for the following clinically relevant genes: *AKT1, AKT3, ALK, ATRX, AURKB, BRAF, C19MC, CCND1, CCND2, CCNE1, CDK4, CDK6, CDKN2A, CDKN2B, CHEK2, CRKL, EGFR, FGF1, FGF2, FGF3, FGF4, FGF5, FGF6, FGF7, FGF8, FGF9, FGF10, FGF11, FGF12, FGF13, FGF14, FGF15, FGF16, FGF17, FGF18, FGF19, FGF20, FGF21, FGF22, FGF23, FGF24, FGF25, FGF26, FGF27, FGF28, FGF29, FGF30, FGF31, FGF32, FGF33, FGF34, FGF35, FGF36, FGF37, FGF38, FGF39, FGF40, FGF41, FGF42, FGF43, FGF44, FGF45, FGF46, FGF47, FGF48, FGF49, FGF50, FGF51, FGF52, FGF53, FGF54, FGF55, FGF56, FGF57, FGF58, FGF59, FGF60, FGF61, FGF62, FGF63, FGF64, FGF65, FGF66, FGF67, FGF68, FGF69, FGF70, FGF71, FGF72, FGF73, FGF74, FGF75, FGF76, FGF77, FGF78, FGF79, FGF80, FGF81, FGF82, FGF83, FGF84, FGF85, FGF86, FGF87, FGF88, FGF89, FGF90, FGF91, FGF92, FGF93, FGF94, FGF95, FGF96, FGF97, FGF98, FGF99, FGF100, FGF101, FGF102, FGF103, FGF104, FGF105, FGF106, FGF107, FGF108, FGF109, FGF110, FGF111, FGF112, FGF113, FGF114, FGF115, 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