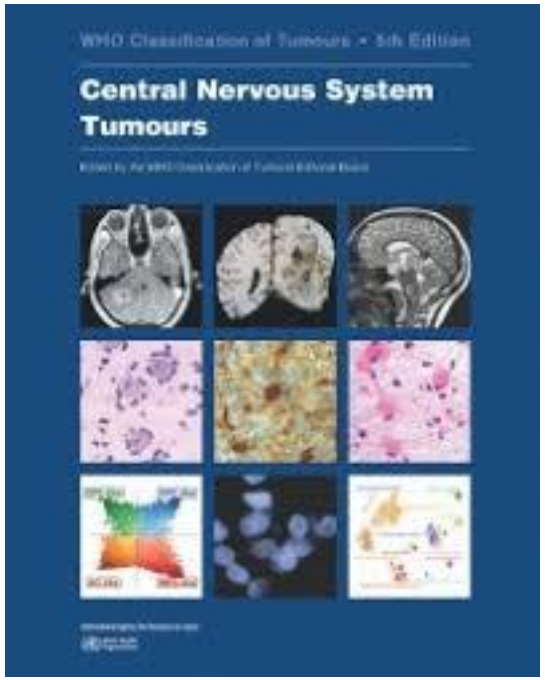
Oxford NANOPORE Technologies

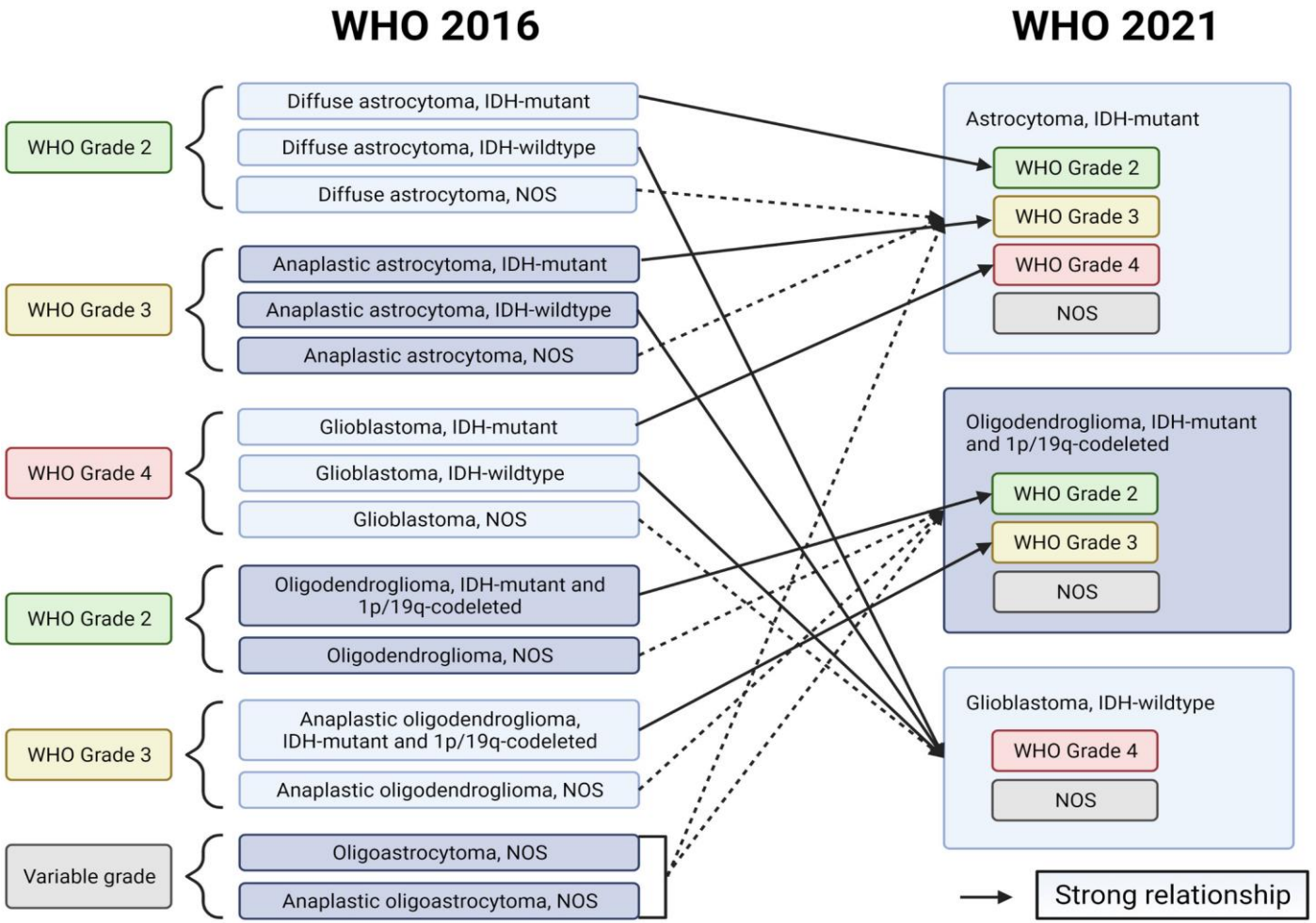
Erfaringer fra St. Olavs Hospital

Anne Jarstein Skjulsvik
Overlege, PhD
Avdeling for patologi

CNS WHO 2021

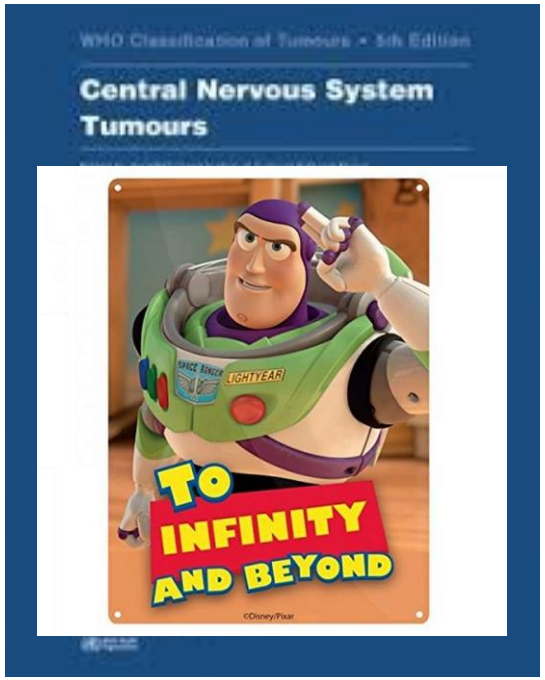


5th



Whitfield BT, Huse JT. Classification of adult-type diffuse gliomas: Impact of the World Health Organization 2021 update. Brain Pathol. 2022 Jul;32(4):e13062. doi: 10.1111/bpa.13062.

CNS WHO 2021

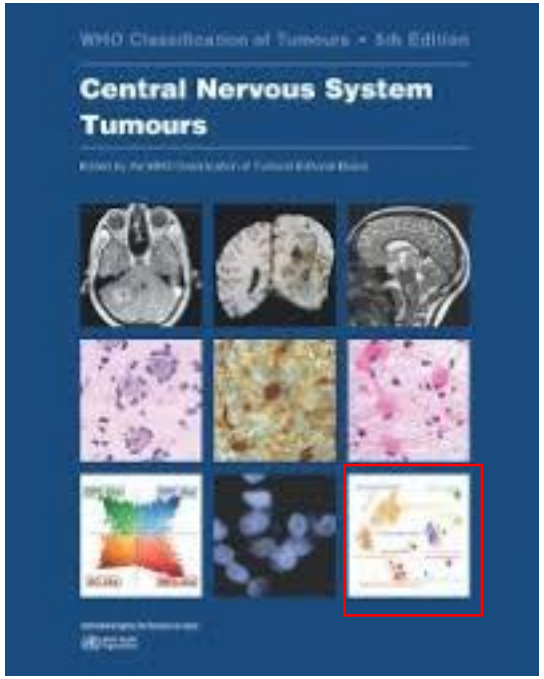


5th

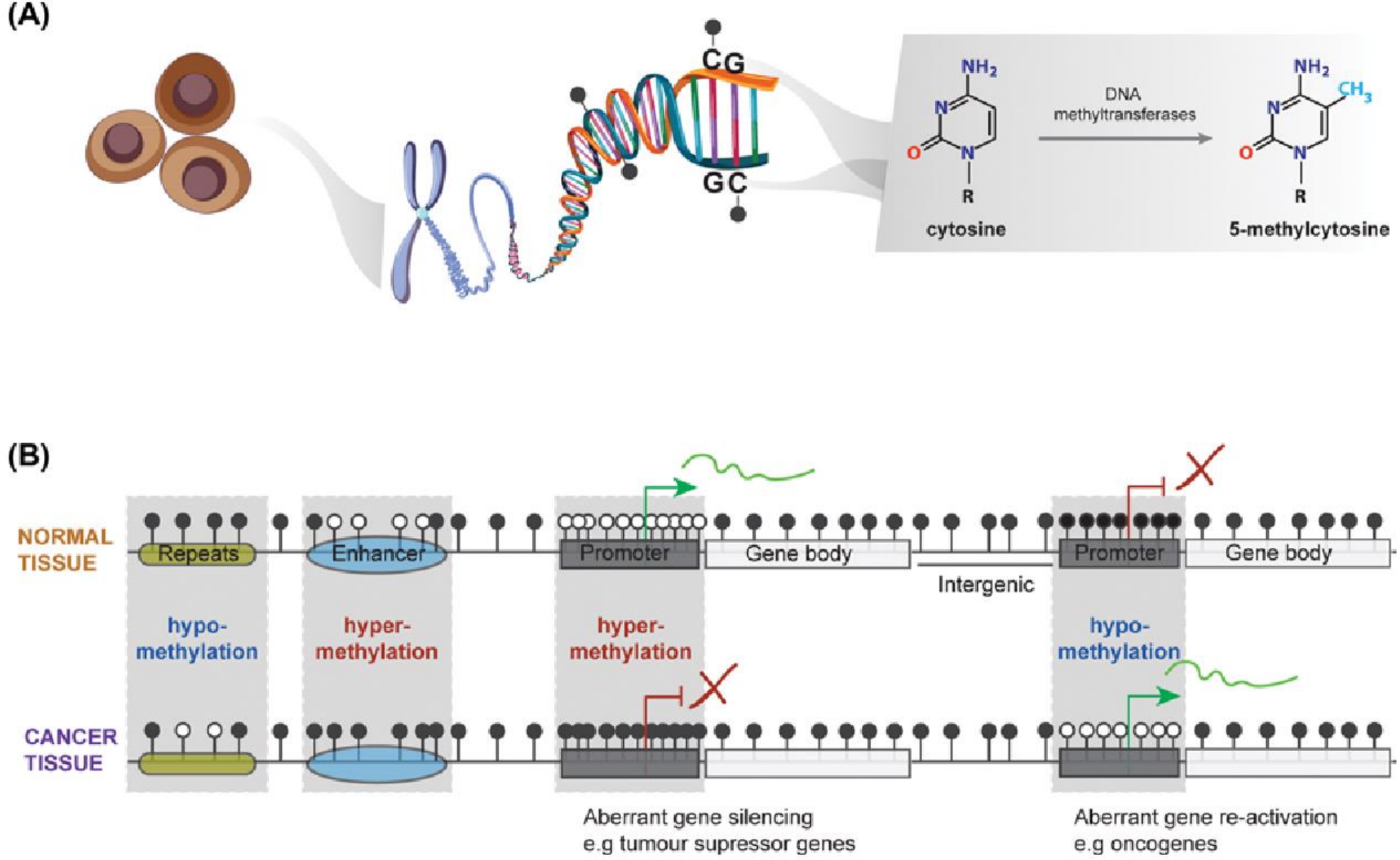
Pediatric-type diffuse low-grade gliomas	Circumscribed astrocytic gliomas	Glioneuronal and neuronal tumors
Diffuse astrocytoma, MYB- or MYBL1-altered	Pilocytic astrocytoma	Ganglioglioma
Angiocentric glioma	**High-grade astrocytoma with piloid features (HGAP)**	Gangliocytoma
Polymorphous low-grade neuroepithelial tumor of the young (PLNTY)	Pleomorphic xanthoastrocytoma (PXA)	Desmoplastic infantile ganglioglioma (DIG)/desmoplastic infantile astrocytoma (DIA)
Diffuse low-grade glioma, MAPK pathway-altered	Subependymal giant cell astrocytoma (SEGA)	Dysembryoplastic neuroepithelial tumor (DNT)
	Chordoid glioma	**Diffuse glioneuronal tumor with oligodendroglioma-like features and nuclear clusters (DGONC)**
	Astroblastoma, MN1-altered	Papillary glioneuronal tumor (PGNT)
		Rosette-forming glioneuronal tumor (RGNT)
		Myxoid glioneuronal tumor (MGNT)
		Diffuse leptomeningeal glioneuronal tumor (DLGNT)
		Multinodular and vacuolating neuronal tumor (MVNT)
		Dysplastic cerebellar gangliocytoma (Lhermitte-Duclos disease)
		Central neurocytoma
		Extraventricular neurocytoma
		Cerebellar liponeurocytoma

In bold, there are highlighted the new entities that have been included in the fifth WHO edition of CNS Tumors.

d'Amati A, Bargiacchi L, Rossi S, Carai A, Bertero L, Barresi V, Errico ME, Buccoliero AM, Asioli S, Marucci G, Del Baldo G, Mastronuzzi A, Miele E, D'Antonio F, Schiavello E, Biassoni V, Massimino M, Gessi M, Antonelli M, Gianno F. Pediatric CNS tumors and 2021 WHO classification: what do oncologists need from pathologists? Front Mol Neurosci. 2024 Mar 13;17:1268038.

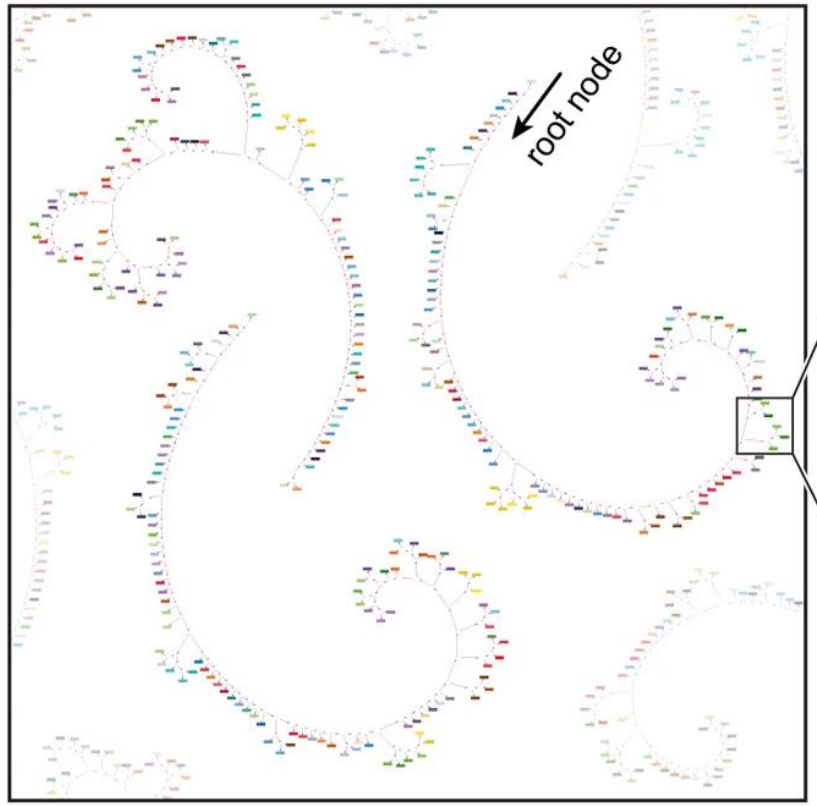


- DNA metylering

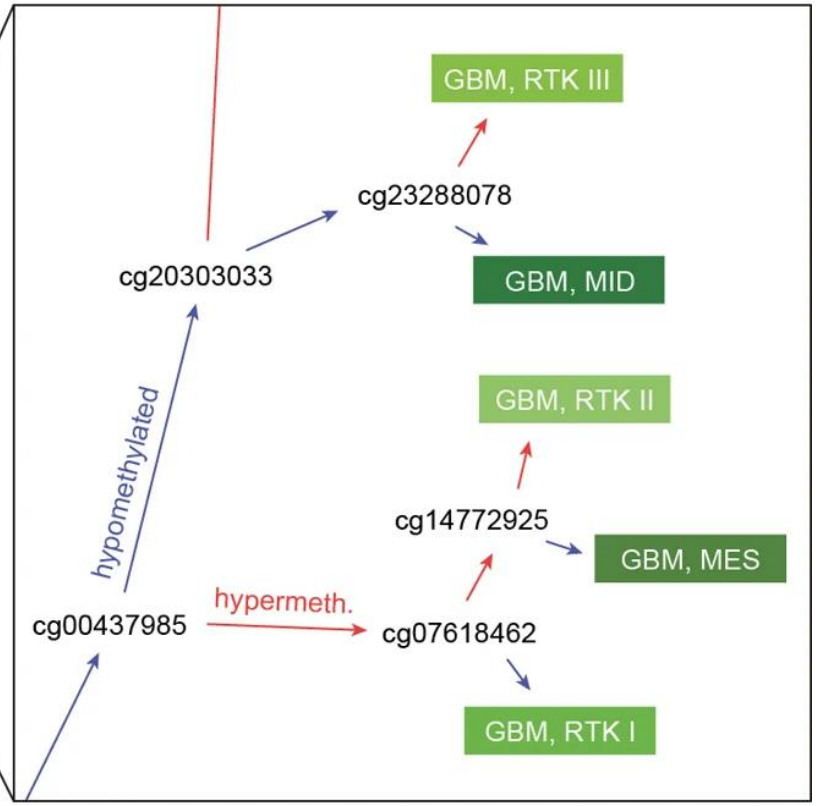


Skvortsova, Ksenia et al. “The DNA methylation landscape in cancer.” Essays in Biochemistry 63 (2019)

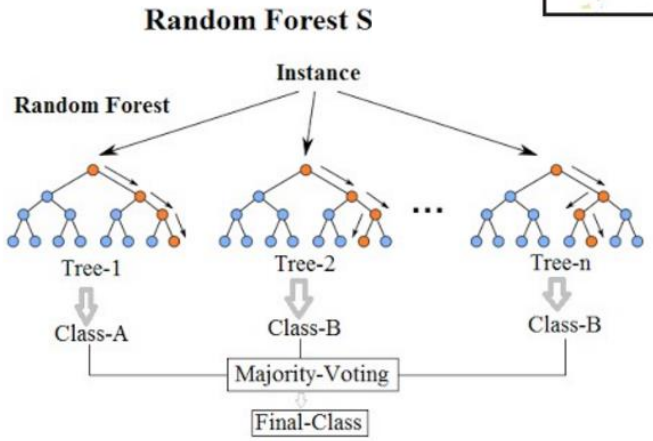
b



3 out of 10,000 decision trees

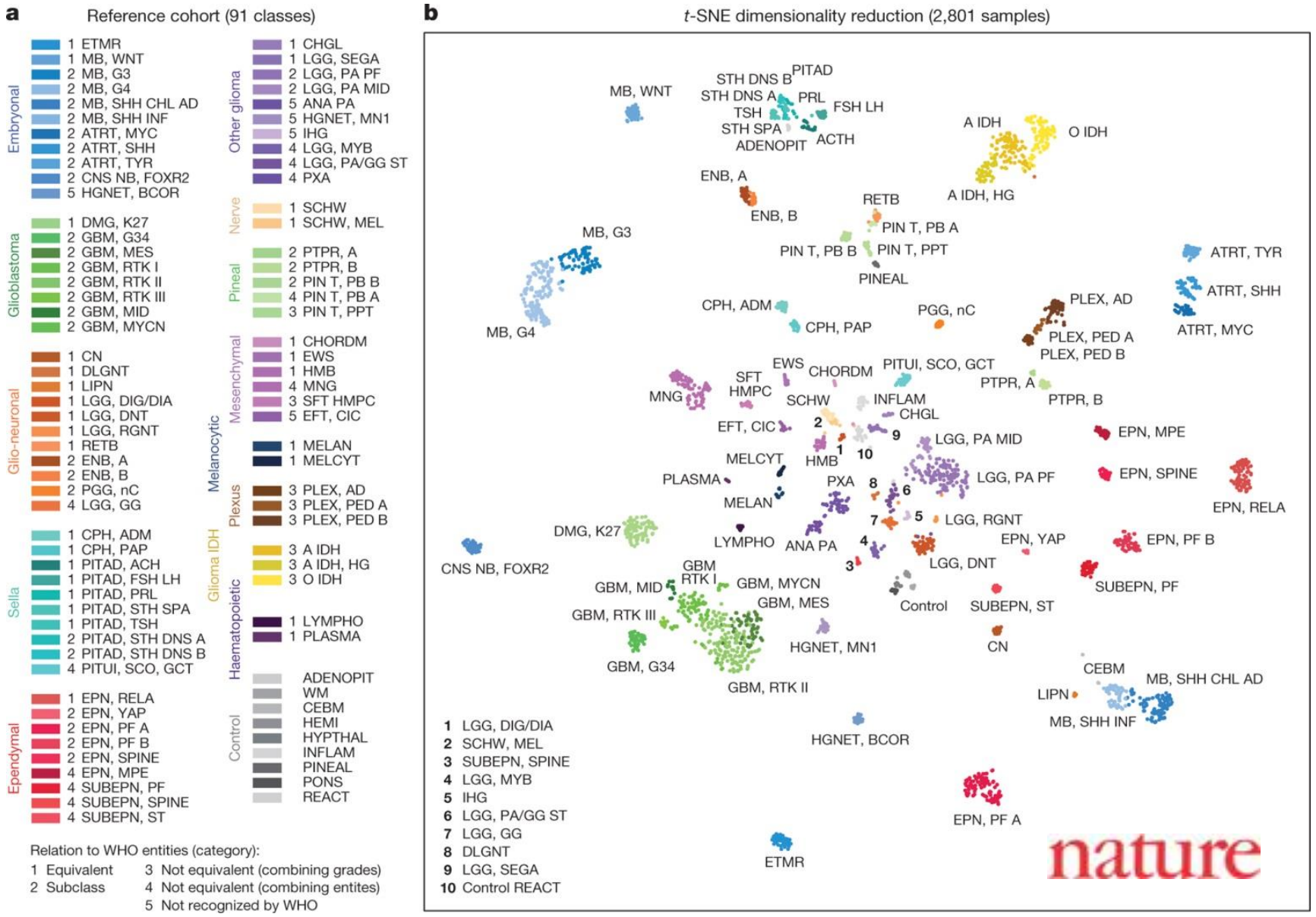


5 decision points & 5 terminal nodes



D Capper *et al. Nature* **555**, 469–474 (2018)
DNA methylation-based classification of central nervous system tumours
PMID: 29539639

Establishing the DNA methylation-based CNS tumour reference cohort



Nasjonalt kvalitets- og kompetansenettverk for solide svulster hos barn (KSSB)

Nasjonalt kvalitets- og kompetansenettverk for solide svulster hos barn (KSSB) er etablert for å bygge opp og spre kompetanse på diagnostikk og behandling av solide svulster hos barn. Med solide svulster menes lokaliserte faste kreftsvulster, i motsetning til for eksempel blodkreft.

EPIC 935K innført i rutine ved St. Olavs Hospital januar 2023

Methylation profiling report

Supplier information

Sample identifier:	
Sentrix ID:	208817100090_R04C01
Material type:	NA
Gender:	NA
Supplier diagnosis:	Anaplastic ependymoma

Automatic prediction		
Array type:	EPICv2	
Material type:	DNA-FFPE	✗
Gender:	unknown	!

Legend: ✓ Ok ! Supplier information or prediction not available ✗ Warning, missmatch of prediction and supplier information

Version 12.8 of the brain classifier results (12.8)

Methylation classes (Highest level >= 0.3, lower levels >= 0.1, all of lowest level)	Calibrated score	Interpretation	
Ependymal Tumours	0.98	match	✓
Supratentorial Ependymoma, Zfta Fusion Positive	0.98	match	✓
Supratentorial Ependymoma, Zfta:rela Fusion Positive	0.95	match	✓
Mc Supratentorial Ependymoma, Zfta Fusion Positive, Subtype Zfta Rela Fused, Subclass A (novel)	0.95	match	✓
Mc Supratentorial Ependymoma, Zfta Fusion Positive, Subtype Zfta Rela Fused, Subclass B (novel)	0.00	no match	✗

Legend: ✓ Match (score >= 0.9) ✗ No match (score < 0.9): possibly still relevant for low tumor content and low DNA quality cases.

Class descriptions

MC Supratentorial ependymoma, ZFTA fusion-positive, subtype ZFTA-RELA fused, subclass A (novel): The "mf Supratentorial ependymoma, ZFTA fusion-positive" includes "mc Supratentorial ependymoma, ZFTA fusion-positive, subtype ZFTA-RELA fused, subclass A", "mc Supratentorial ependymoma, ZFTA fusion-positive, subtype ZFTA-RELA fused, subclass B", "mc Supratentorial ependymoma, ZFTA fusion-positive, subclass C", "mc Supratentorial ependymoma, ZFTA fusion-positive, subclass D" and "mc Supratentorial ependymoma, ZFTA fusion-positive, subclass E", and represents the most common group of supratentorial pediatric ependymomas. Within this mf, subclasses A and B typically show the canonical ZFTA:RELA fusion.

MC Supratentorial ependymoma, ZFTA fusion-positive, subtype ZFTA-RELA fused, subclass B (novel): The "mf Supratentorial ependymoma, ZFTA fusion-positive" includes "mc Supratentorial ependymoma, ZFTA fusion-positive, subtype ZFTA-RELA fused, subclass A", "mc Supratentorial ependymoma, ZFTA fusion-positive, subtype ZFTA-RELA fused, subclass B", "mc Supratentorial ependymoma, ZFTA fusion-positive, subclass C", "mc Supratentorial ependymoma, ZFTA fusion-positive, subclass D" and "mc Supratentorial ependymoma, ZFTA fusion-positive, subclass E", and represents the most common group of supratentorial pediatric ependymomas. Within this mf, subclasses A and B typically show the canonical ZFTA:RELA fusion.

Proficiency Test Report

DNA Methylation Array 935 K / Brain Tumor Classifier (MolPath, Neuropathology) 2023

FHI | Mini-metodevurdering - *for vurdering av tiltak i sykehus og kommuner*

Forside › Mini-metodevurderinger › DNA metylering av kreftsvulster ...

Søk etter mini-metodevurderinger

Meny

DNA metylering av kreftsvulster

Publisert 22.08.2024 | Sist oppdatert 23.08.2024

Kontakt forslagsstiller (Hege Elisabeth Giercksky Russnes) Del med andre Skriv ut

Fagområde
Kunstig intelligens og digitale løsninger
Kreftsykdommer

Metodetype
Diagnostisk metode

Helseforetak
Oslo Universitetssykehus

Publisert
2024

MEN..

Sekvensatorer og flowceller

MinION

The Flongle Flow Cell can generate up to 2.8 Gb of data enabling direct, real-time DNA & cDNA sequencing on smaller, single-use flow cells.

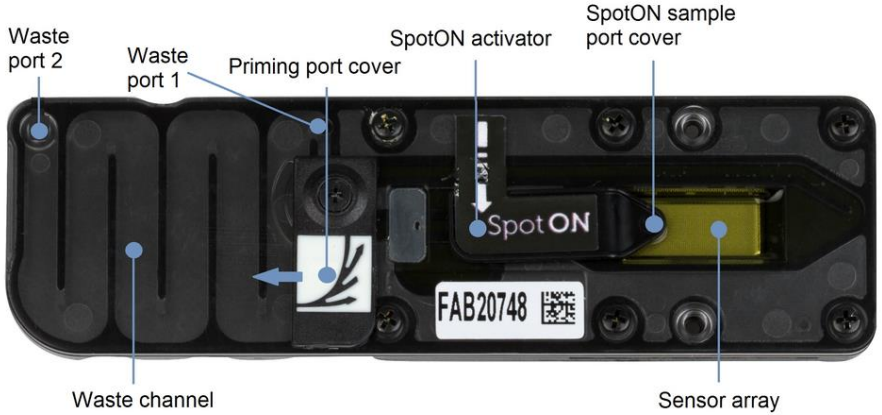
GridION

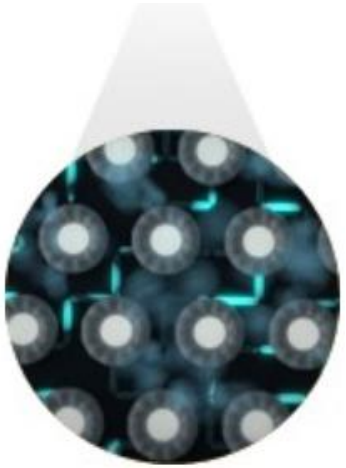
The MinION Flow Cell can generate up to 50 Gb of data for sequencing DNA, cDNA or native RNA in real-time.

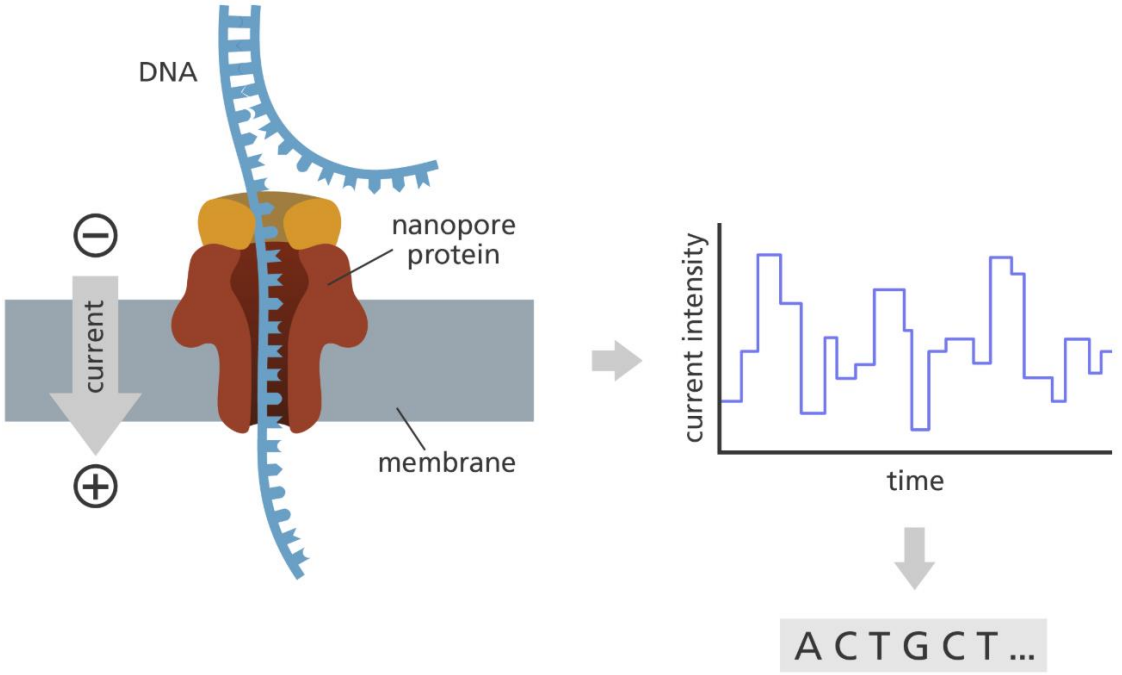
PromethION

The PromethION Flow Cell can generate up to 290 Gb for sequencing DNA, cDNA or native RNA in real-time.

MinION flowcelle

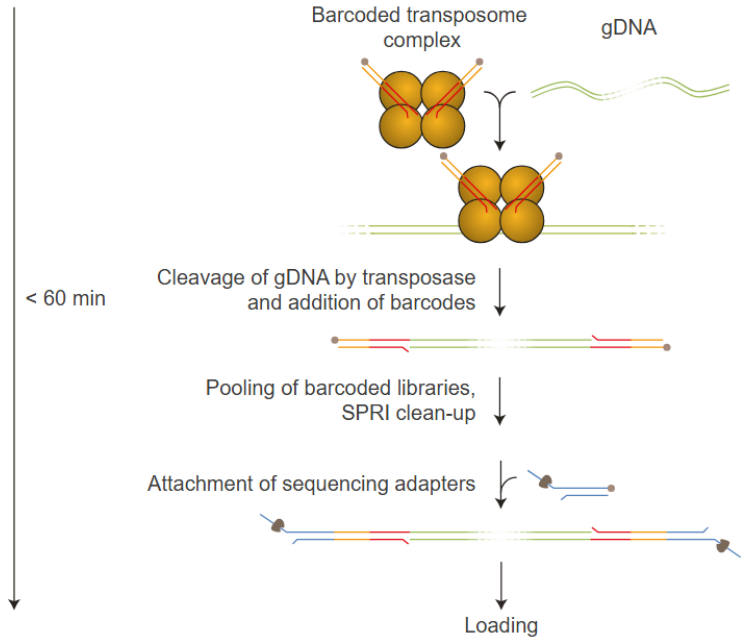




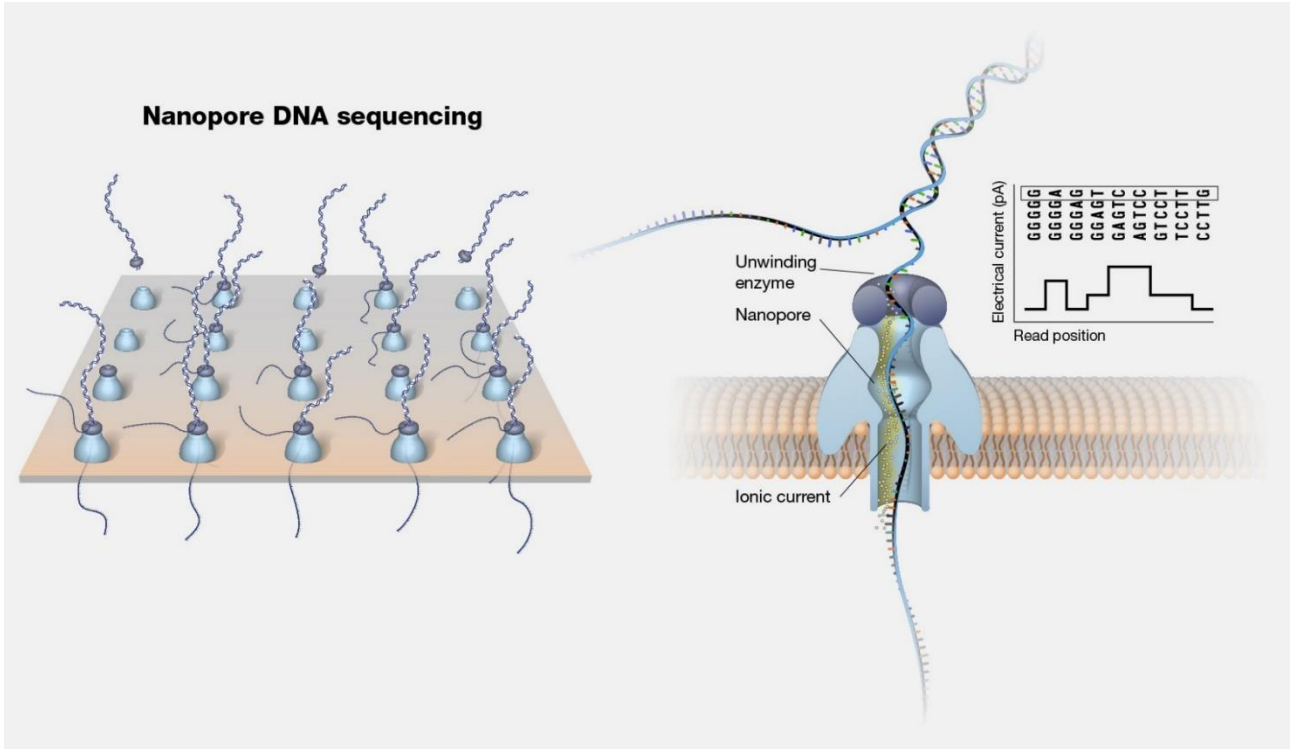


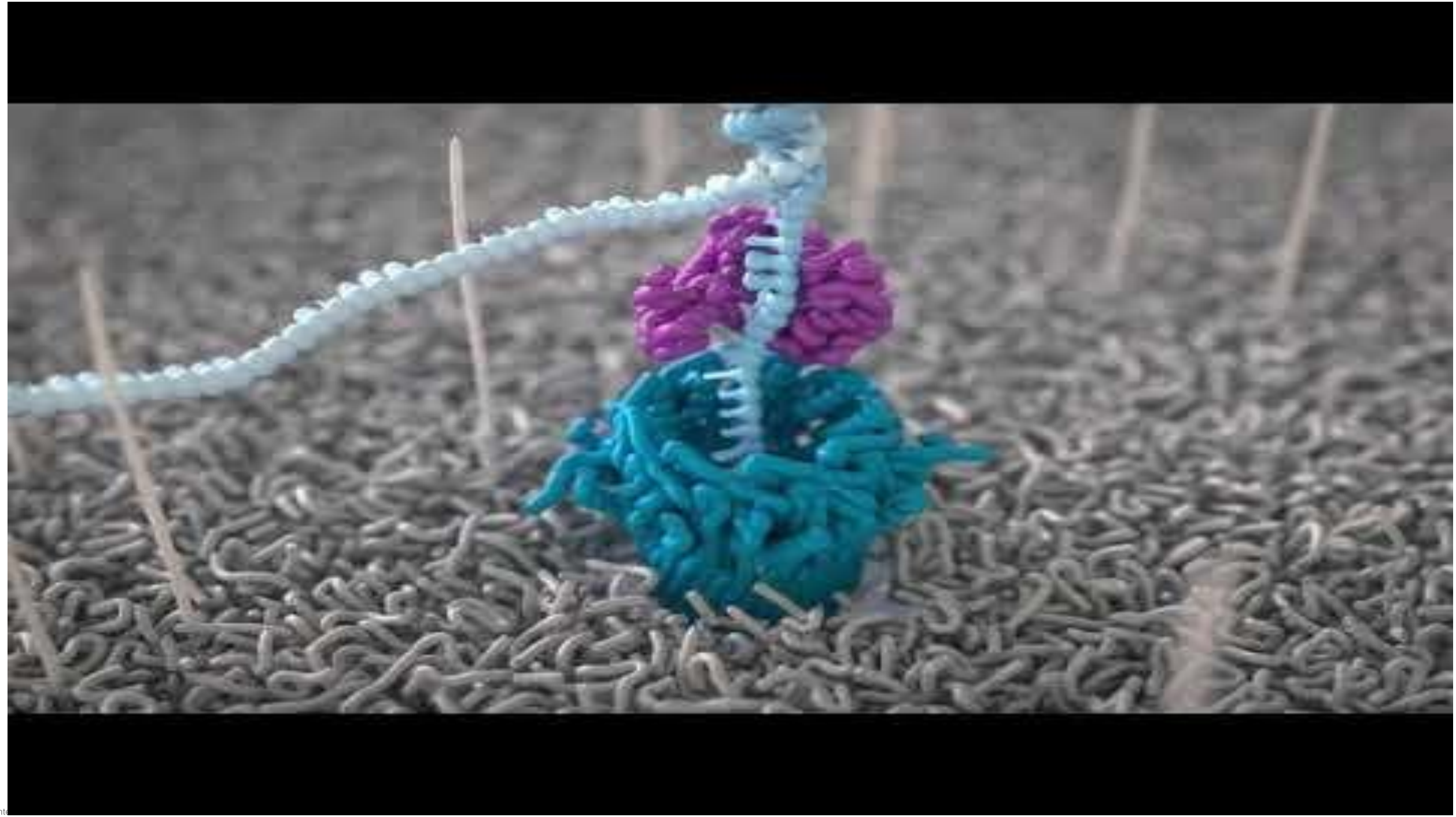
Nanopore-sekvensering

Library prep



Sekvensering





Hva brukes metoden til?

Acta Neuropathol (2017) 134:691–703
DOI 10.1007/s00401-017-1743-5

METHODS PAPER

Same-day genomic and epigenomic diagnosis of brain tumors using real-time nanopore sequencing

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DOI: 10.1111/nan.12856

ORIGINAL ARTICLE

Neuropathology and Applied Neurobiology
WILEY

Robust methylation-based classification of brain tumours using nanopore sequencing

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Article

Ultra-fast deep-learned CNS tumour classification during surgery

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Check for updates

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Central nervous system tumours represent one of the most lethal cancer types, particularly among children[1]. Primary treatment includes neurosurgical resection of the tumour, in which a delicate balance must be struck between maximizing the extent of resection and minimizing risk of neurological damage and comorbidity[2,3]. However, surgeons have limited knowledge of the precise tumour type prior to surgery. Current standard practice relies on preoperative imaging and intraoperative histological analysis, but these are not always conclusive and occasionally wrong. Using rapid nanopore sequencing, a sparse methylation profile can be obtained during surgery[4]. Here we developed Sturgeon, a patient-agnostic transfer-learned neural network, to enable molecular subclassification of central nervous system tumours based on such sparse profiles. Sturgeon delivered an accurate diagnosis within 40 minutes after starting sequencing in 45 out of 50 retrospectively sequenced samples (abstaining from diagnosis of the other 5 samples). Furthermore, we demonstrated its applicability in real time during 25 surgeries, achieving a diagnostic turnaround time of less than 90 min. Of these, 18 (72%) diagnoses were correct and 7 did not reach the required confidence threshold. We conclude that machine-learned diagnosis based on low-cost intraoperative sequencing can assist neurosurgical decision-making, potentially preventing neurological comorbidity and avoiding additional surgeries.

Ulike klassifiseringsverktøy/ maskinlæringsalgoritmer for analysering av data

- NanoDX
- Sturgeon
- ROBIN
- MNP-FLEX
- …

Gullstandard er array-basert DNA-metyleringsanalyse EPIC 935K (METYL_EPIC)

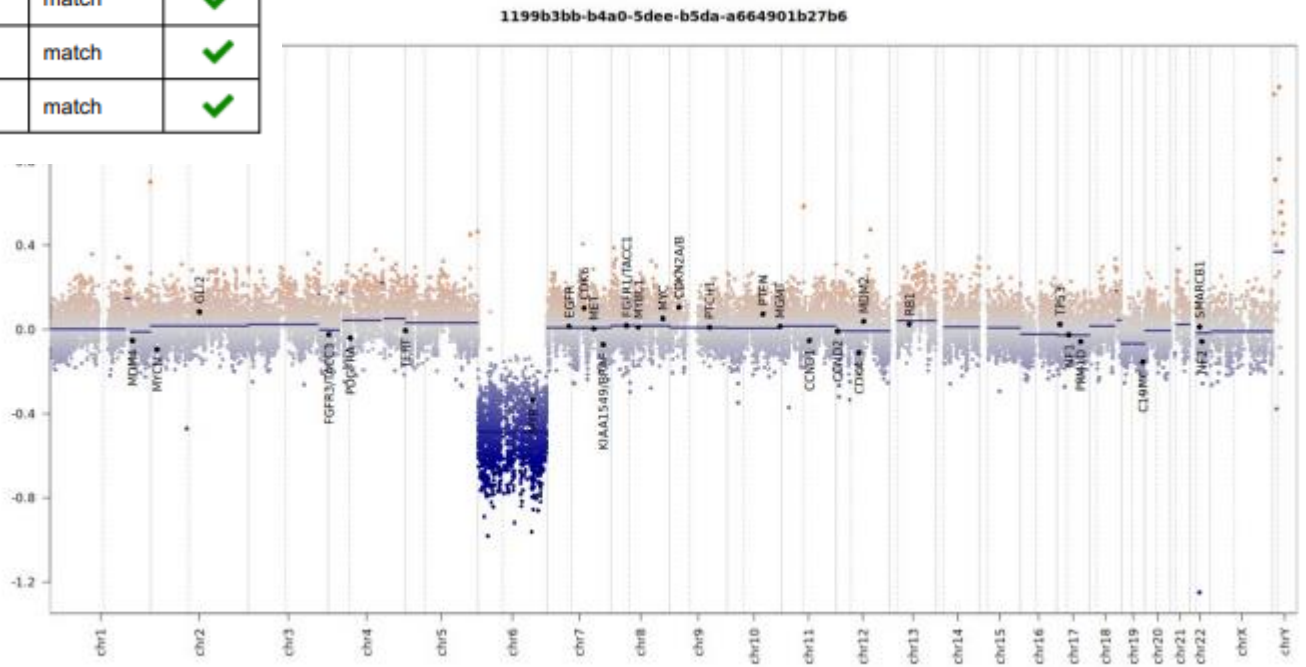
Nasjonalt kvalitets- og kompetansenettverk for solide svulster hos barn (KSSB)

Nasjonalt kvalitets- og kompetansenettverk for solide svulster hos barn (KSSB) er etablert for å bygge opp og spre kompetanse på diagnostikk og behandling av solide svulster hos barn. Med solide svulster menes lokaliserte faste kreftsvulster, i motsetning til for eksempel blodkreft.

SFH7-25:
EPIC 935K: 1,0
Medulloblastoma WNT

Methylation classification

Predictions	Calibrated score	Interpretation	
Medulloblastoma	1.0	match	✔
medulloblastoma, WNT activated	1.0	match	✔
medulloblastoma, WNT activated	1.0	match	✔
MC Medulloblastoma, WNT-activated	1.0	match	✔

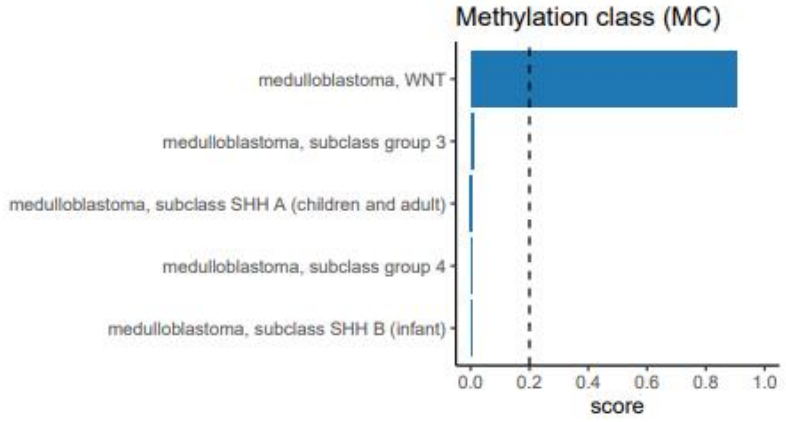


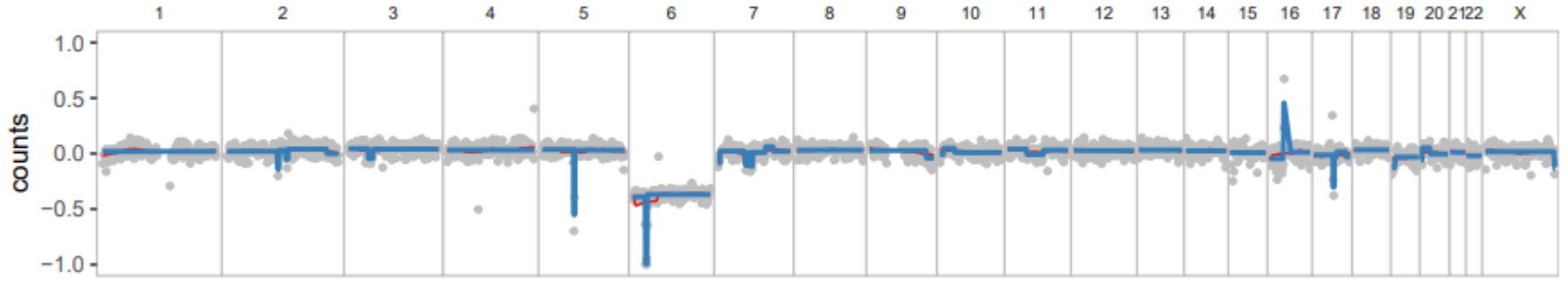
SFH7-25
NanoDx 0,9 Medulloblastoma WNT

Methylation-based classification

Methylation-based classification is based on **275350** CpG sites (overlap of sites covered in this sample and the model). At the methylation class (MC) level, the sample has been classified as **medulloblastoma, WNT**. This prediction has a confidence score of **0.904**. At the methylation class **family** (MCF) level, the sample has been classified as **medulloblastoma, WNT**. The MCF prediction has a confidence score of **0.904**.

Scores for the Top 5 entities on MC and MCF level are given below. Vertical dashed lines indicate the recommended >0.2 cut-off for classification.





	NanoDx Single Sample 90 min	NanoDx Single sample End of life	NanoDx Single sample 24h	NanoDx multiplex 90min	NanoDx multiplex 24h	NanoDx Adaptive sampling CpG 40min	NanoDx Adaptive sampling Cpg 3h	
SFH07-25			0,904	-	-	-	-	Medulloblastoma Wnt-activated
SFH08-25			0,803	-	-	0,773	0,792	Medulloblastoma Subclass group 3-4
SFH09-25			-	0,277	0,251	0,255	0,324	Glioblastoma, RTK1 or RTK2
SFH10-25			-	0,317	0,539	0,543	0,563	Glioblastoma, RTK2
SFH12-25			-	0,507	0,687	0,272	0,506	Oligodendro-glioma
SFH16-25			-	0,811	0,907	-	-	Meningeoma
SFH17-25	0,303	0,392						Glioblastoma Mesenchymal*
SFH19-25	0,352	0,324						Astrocytoma, IDHmut

Nanoporesekvensering for DNA-metylering

- Metoden er testet og validert
- Bygget IT-infrastruktur for håndtering av data
- Logistikk

Metoden er nå tatt i bruk i rutine ved St. Olavs Hospital fra september 2025

Prøvesvaret integreres i svarrapport fra patologen på lik linje med andre analyser (immunhistokjemi, PCR, genpanel, FISH)

Planlegger utvidet bruk (sarkomer, cancer ukjent origo)

Takk til

Nasjonalt kvalitets- og kompetansenettverk for solide svulster hos barn (KSSB)

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