# Methylation profiling for CNS tumor classification

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MB&C course 06/02/2026

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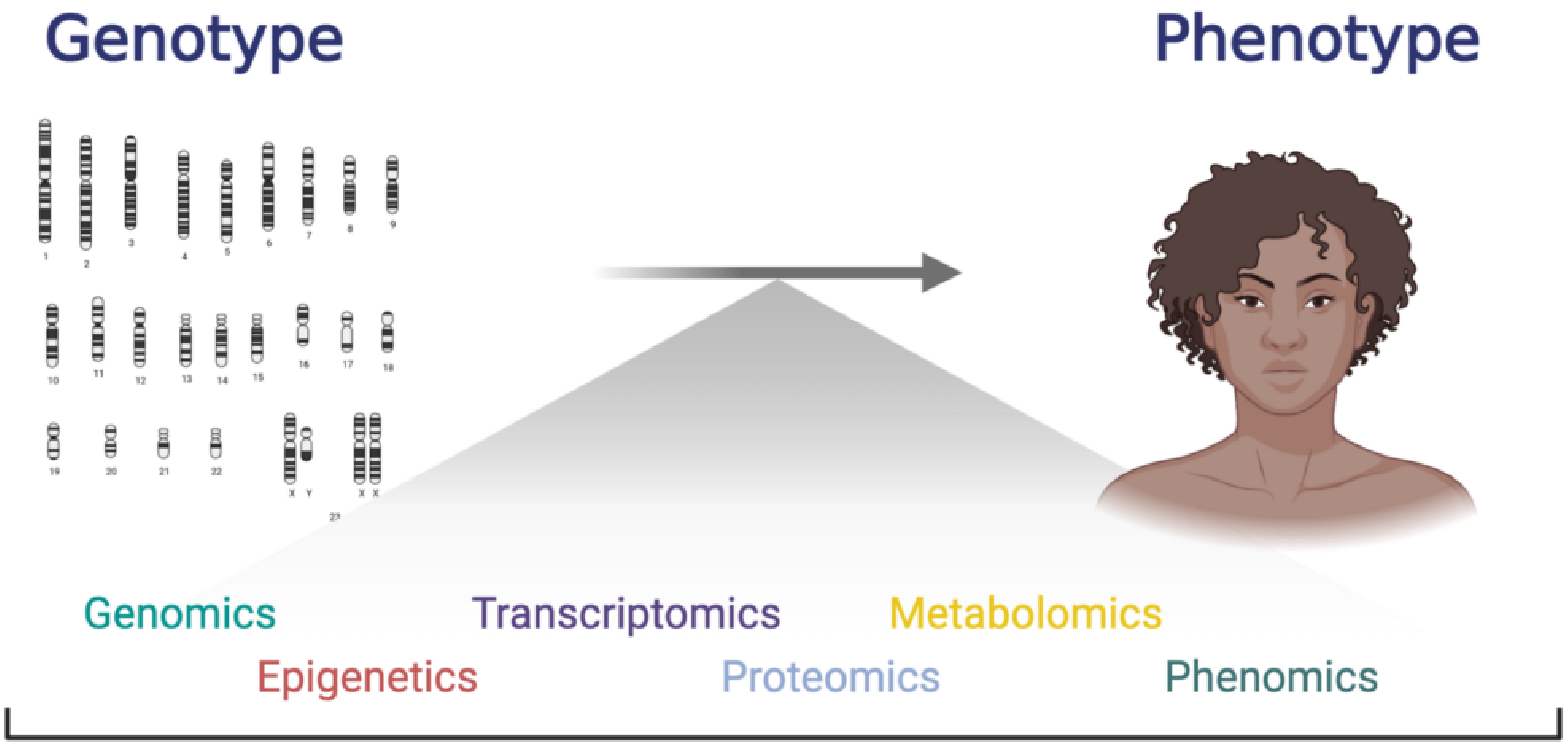
# Methylation profiling for CNS tumor classification

- Introduction
  - Epigenitics
  - Methylation
- DNA methylation detection methods
- Methylation for diagnostics
- Methylation classifiers for CNS tumors
  - MNP
  - Rapid CNS$^2$
  - Sturgeon
  - MNP-Flex

# Omics to bridge the genotype – phenotype gap



**Genotype**

**Phenotype**

Genomics

Transcriptomics

Metabolomics

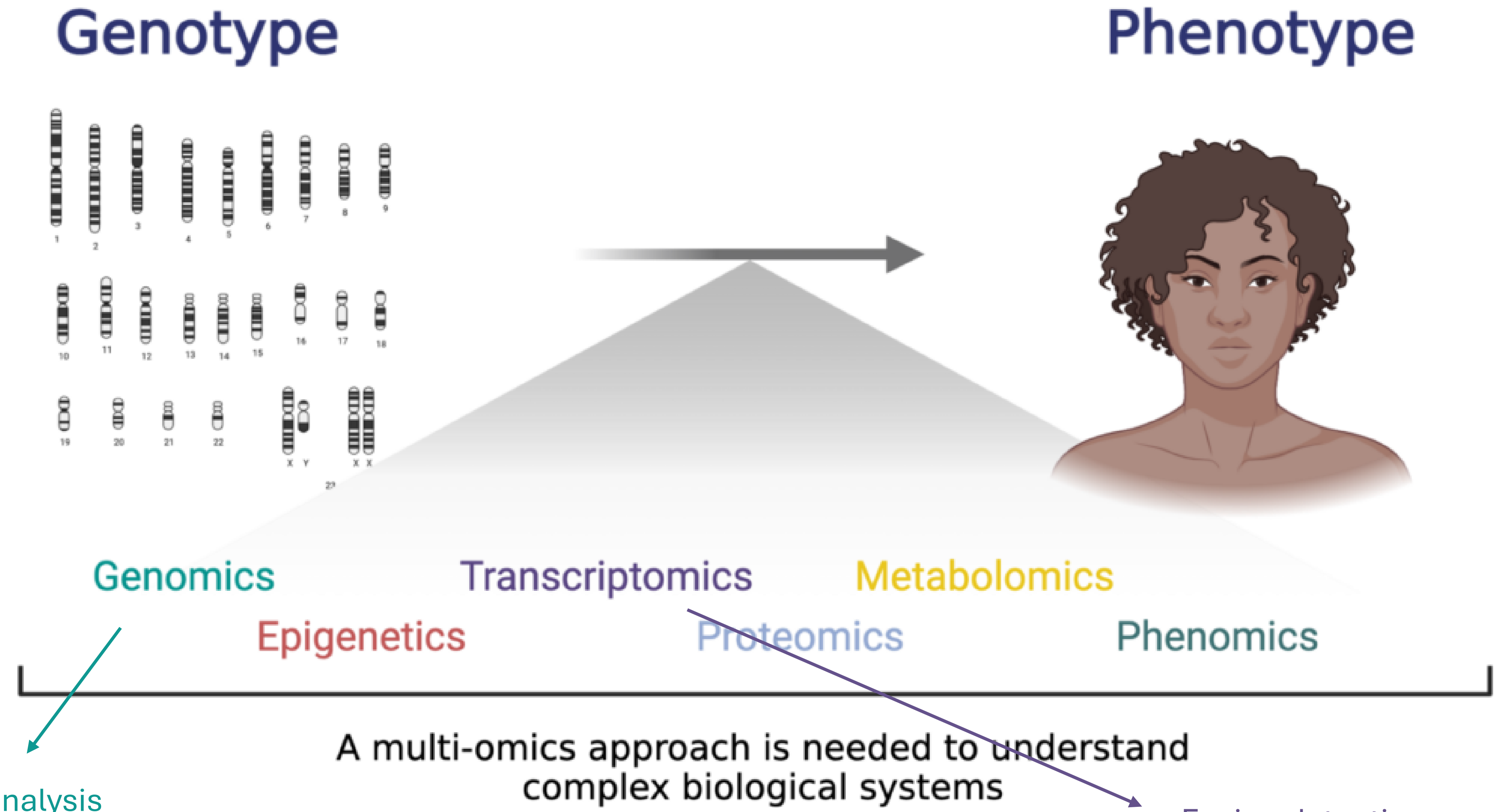
Epigenetics

Proteomics

Phenomics

A multi-omics approach is needed to understand complex biological systems

# Omics to bridge the genotype – phenotype gap



A multi-omics approach is needed to understand complex biological systems

- Mutation analysis
- CNV detection

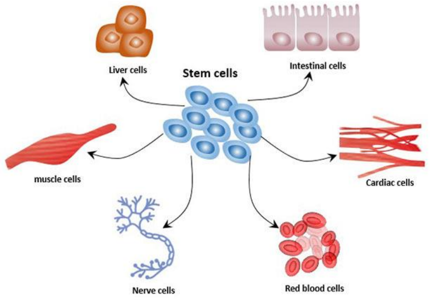
- Fusion detection
- Expression analysis

# Epigenetics

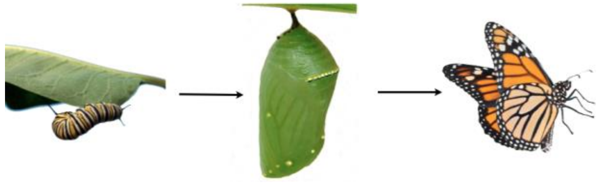
- modifications **influence gene activity** but do **not** change the actual genetic code (the A, T, C, G sequence).
- The Greek prefix epi- (ἐπι): "over, outside of, around"

→features that are "on top of" or "in addition to" the traditional DNA-sequence-based mechanism of inheritance

- Epigenetics
  - Presist through cell division
  - Important during normal development > differentiation
  - influenced by environmental factors



Epigenetic changes orchestrate differentiation

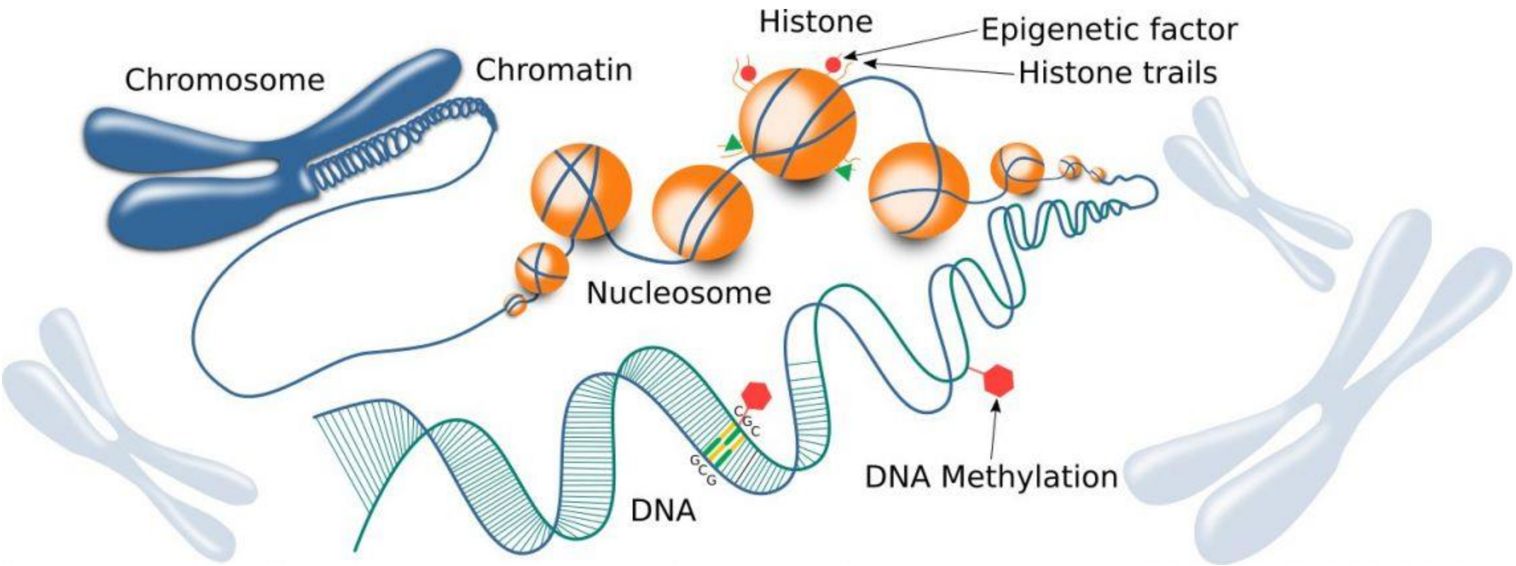


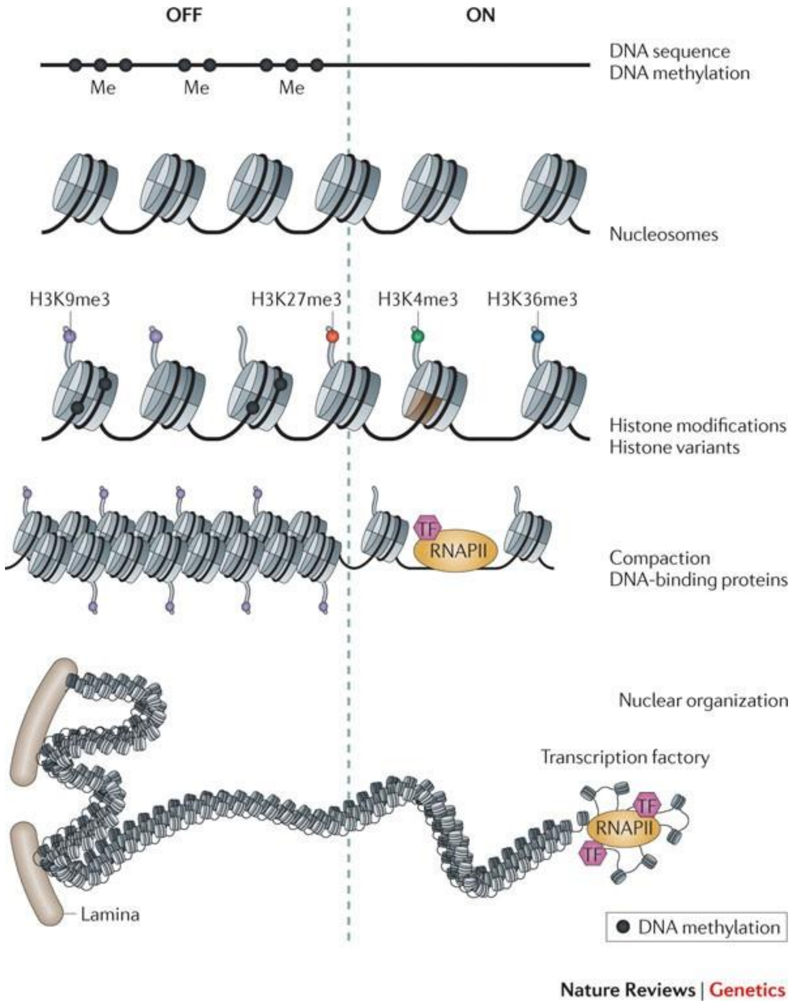
Butterfly metamorphosis – same genes

Epigenetic effects from dutch famine 1944-1945

# Epigenetics

- **Epigenetic modifications** affect how tightly DNA is packed and how accessible it is to transcription machinery.
- Includes:
  - Histone Modifications
  - DNA Methylation
  - Chromatin Remodeling
  - Non-coding RNAs

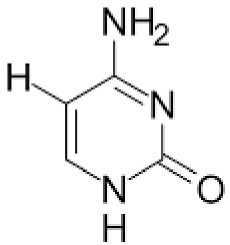




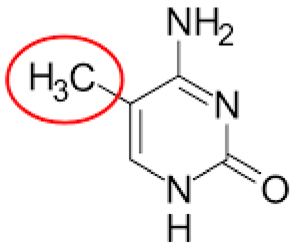
Zhou et al., Nature reviews 2010

# DNA methylation across the genome

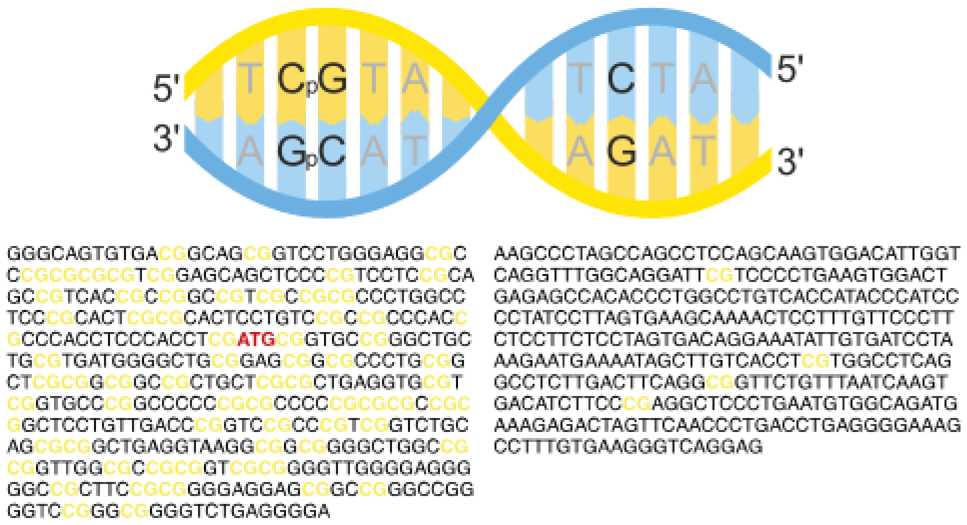
- involving the transfer of a methyl group onto the C5 position of the cytosine to form 5-methylcytosine.
- 70-80% of methylation at CpG
- high frequency of CpG sites: CpG islands



Cytosine



Methylated Cytosine

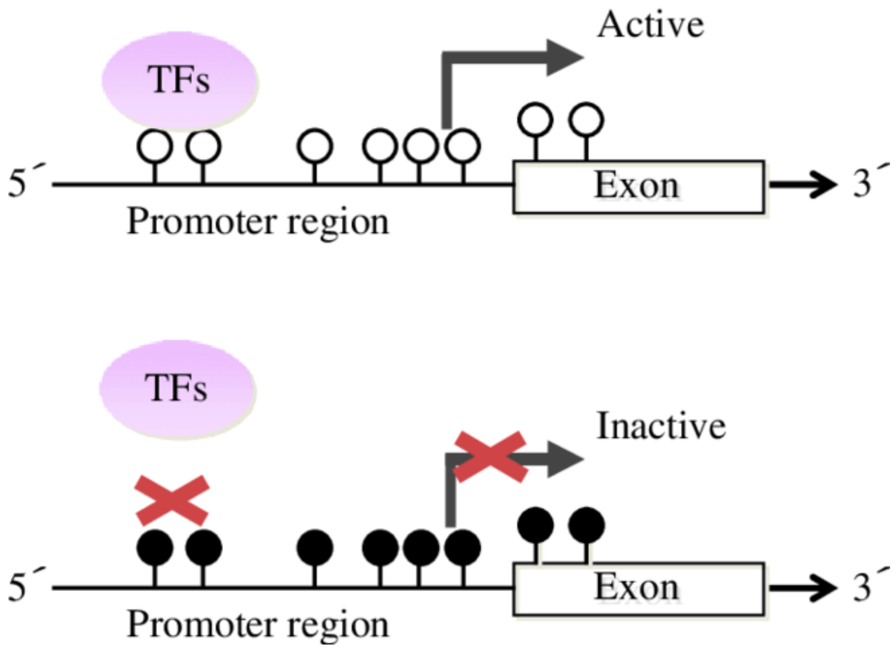


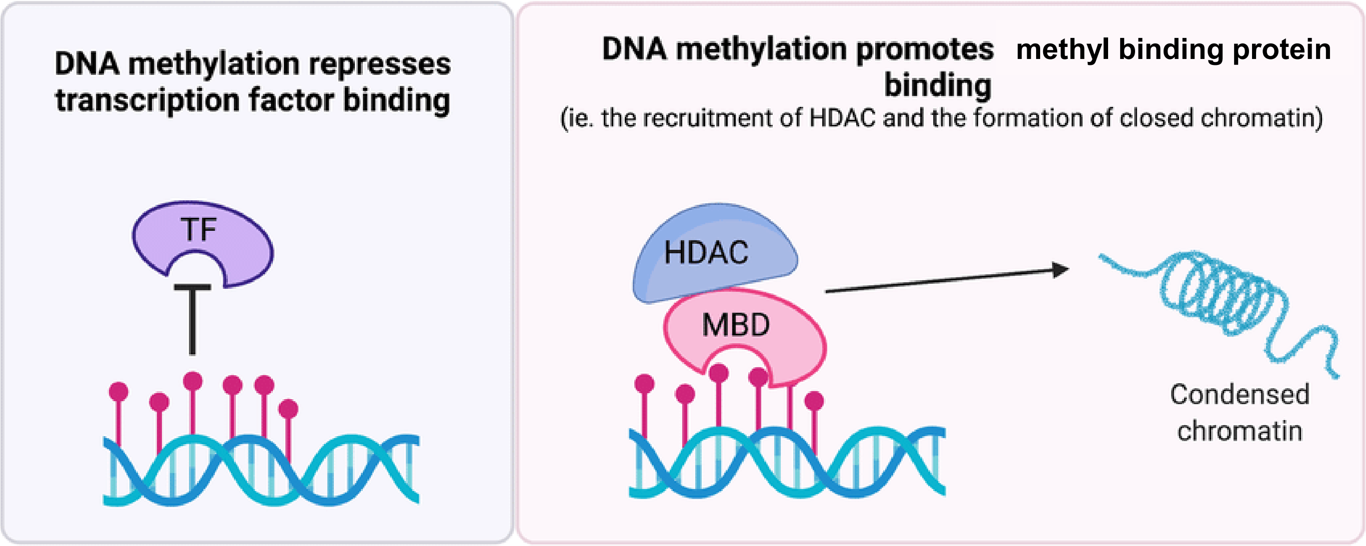
CpG islands
>60% CpG
Promoter region

CpG at other
region in genome

# DNA methylation regulates gene expression

- inhibiting the binding of transcription factor(s) to DNA
- recruiting proteins involved in gene repression: methyl-CpG-binding domain (MBD)
- Cross talk with other epigenetic mechanisms like histone modifications

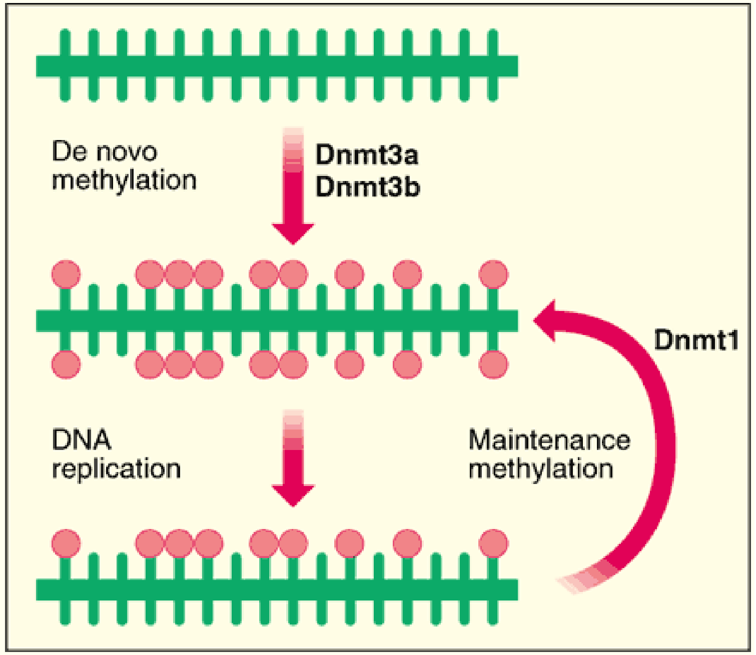


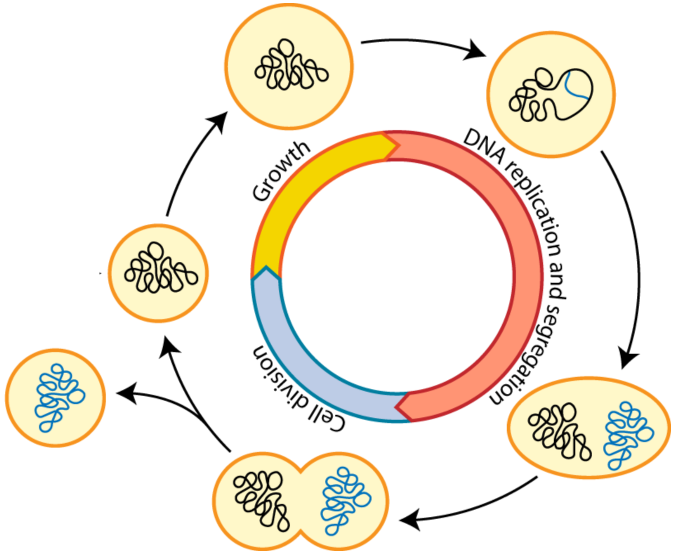

DNA methylation represses transcription factor binding

DNA methylation promotes methyl binding protein binding
(ie. the recruitment of HDAC and the formation of closed chromatin)

# DNA methylation is performed by DNA methyltransferases (DNMTs)

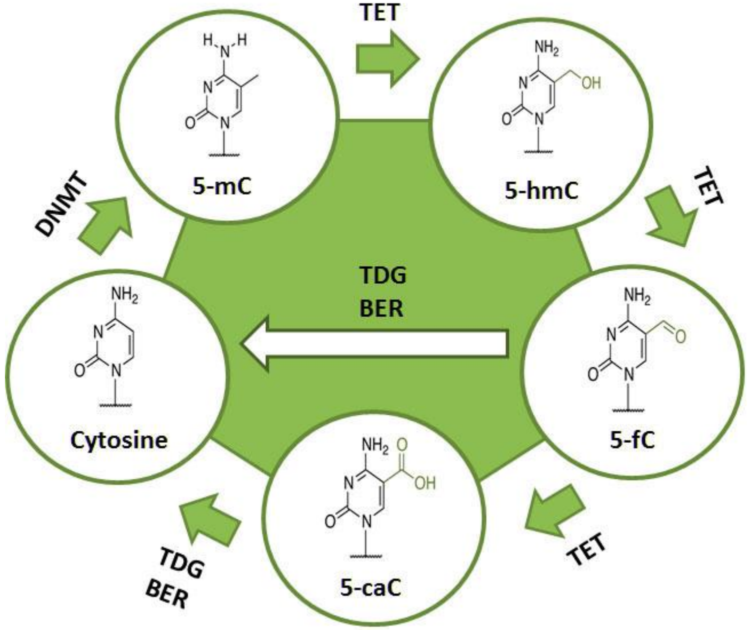
- De Novo methylation: DNMT3a/b
    - add methyl groups to previously unmethylated cytosine bases
    - Important for embryonic development, differentiation, genomic imprinting
- Maintenance methylation: DNMT1
    - Copy methylation pattern of parental strand during DNA replication





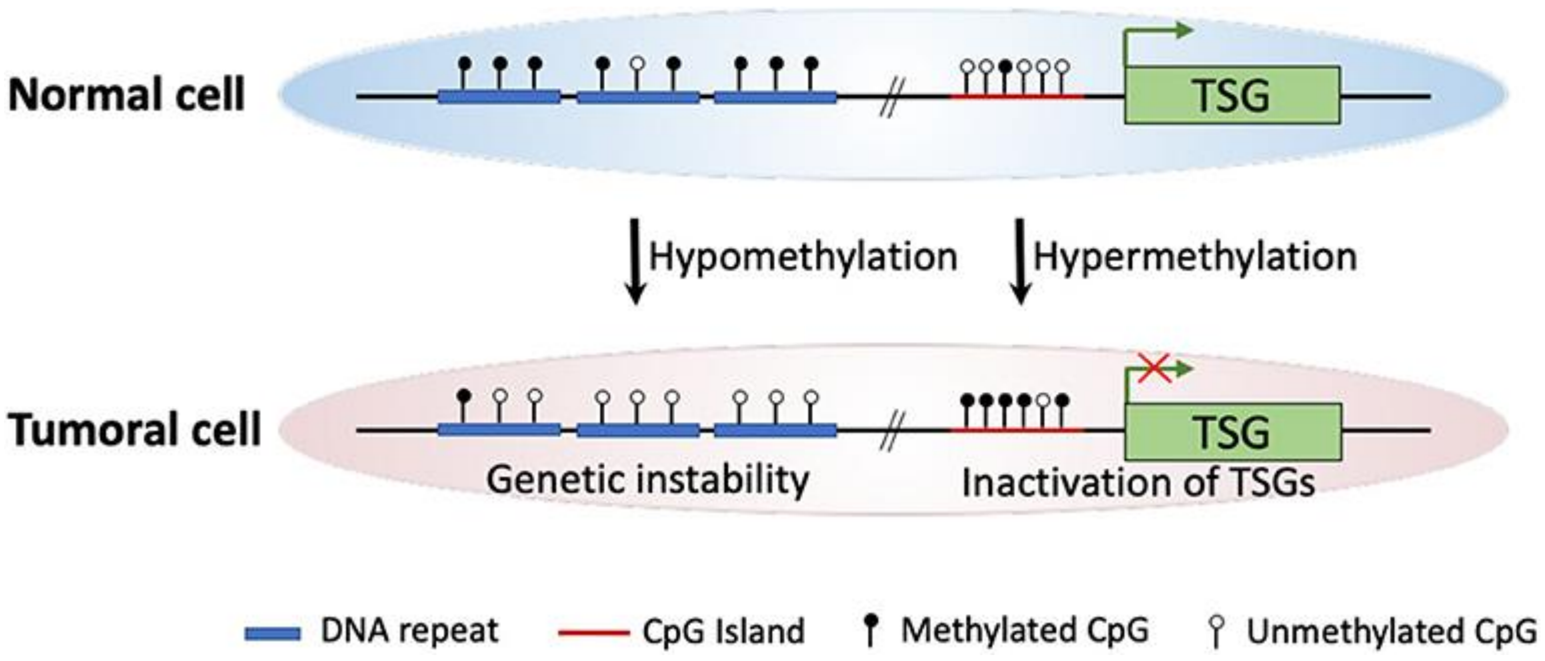
# DNA demethylation

- DNA demethylation
  - Active: TET enzymes
    - Through 5hmC intermediate
    - Followed by base excision repair
  - Passive: preventing addition methyl group during replication
  - Important during: cellular repair, neuronal function & memory, embryonic development,…



# DNA Methylation in Cancer

- Disruption of DNA methylation can lead to cancer. For example: mutations in DNMT’s
  - Global hypomethylation → genetic instability
  - Hypermethylation of promoter regions of tumor suppressor genes



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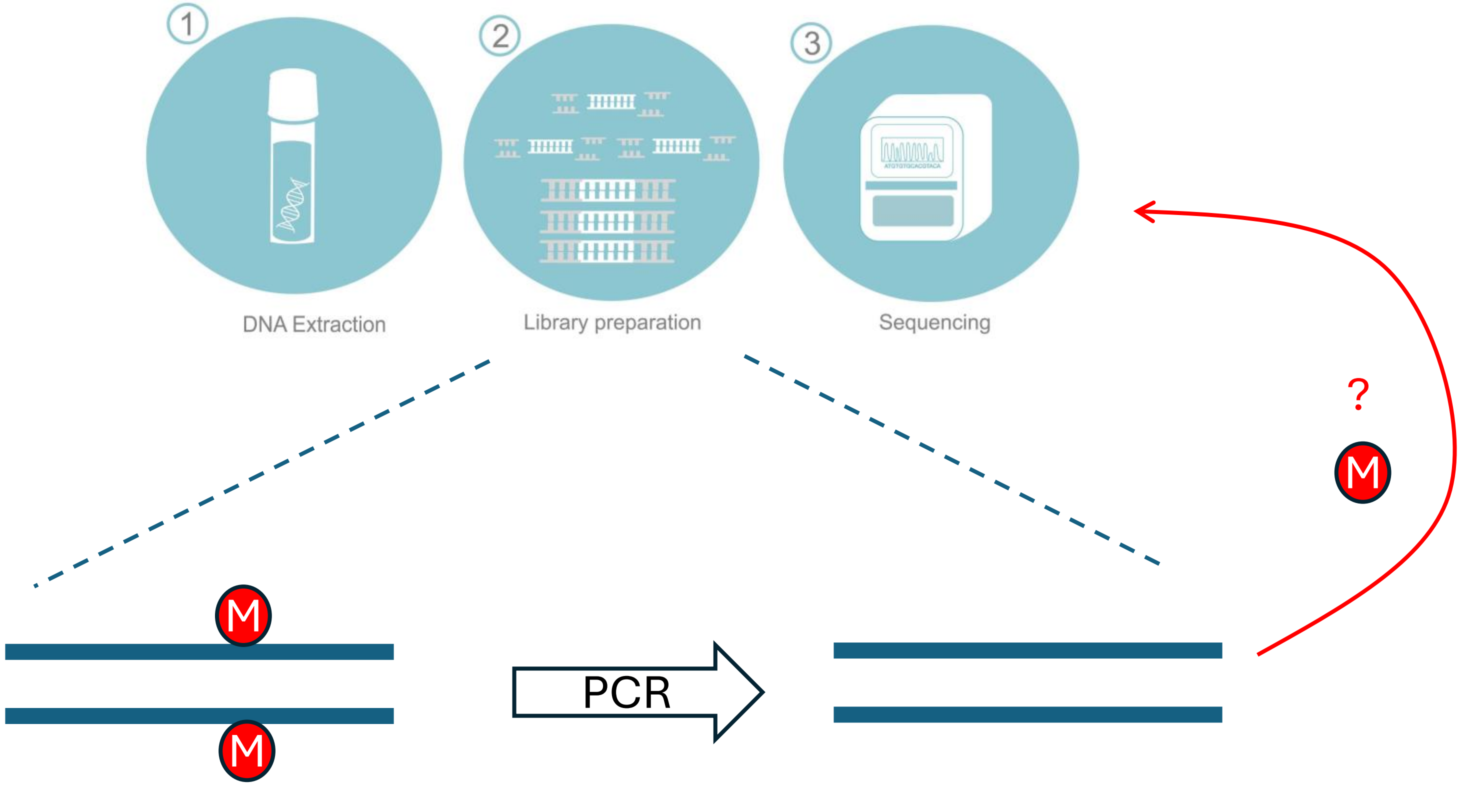
# How to detect DNA methylation?

A) Bisulfite based methods
B) Enrichment based sequencing methods
C) Enzymatic methyl sequencing
D) 3th generation sequencing

# Bisulfite based methods

Bisulfite conversion is used to retain the methylation status during sequecing



# Bisulfite based methods

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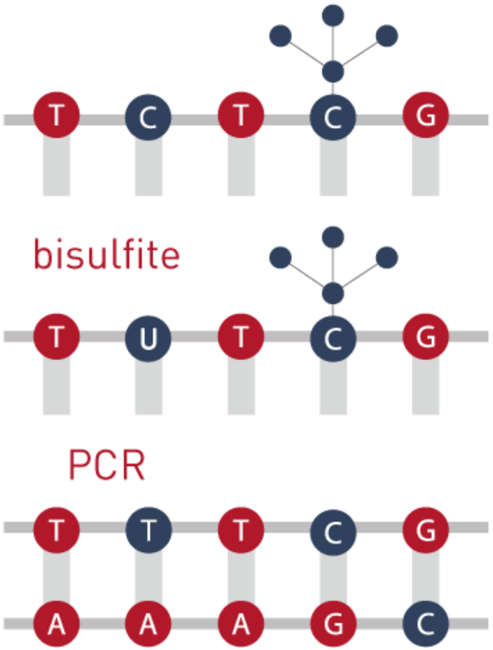
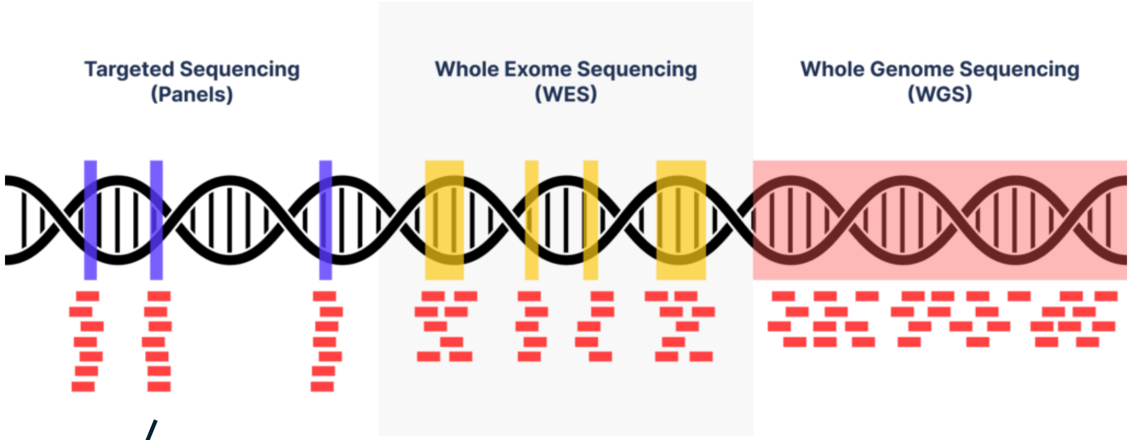


**Unmethylated** cytosines (C) are chemically converted into uracils (U)

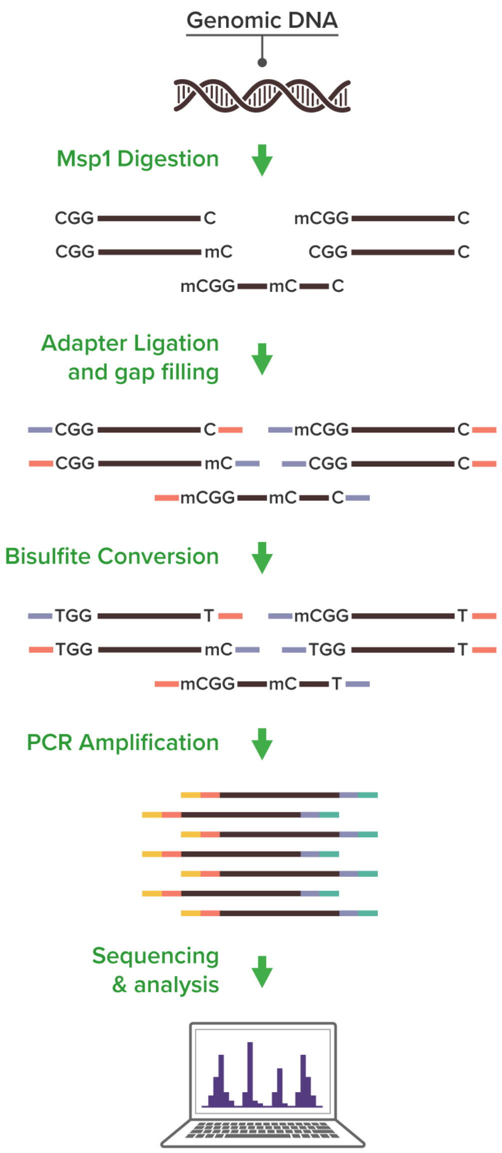
Uracils (U) are amplified as thymines (T) during PCR

# Bisulfite based methods

- Whole genome bisulfite sequecing
- Targetted bisulfite sequencing
- Reduced Representation Bisulfite sequening (RRBS)
- Array based methods



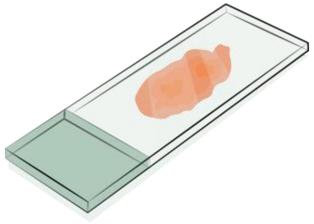
! Methylation specific probes



RRBS

# Illumina EPIC array

- 450K/930K unique methylation sites
- Measure methylation at **predefined** CpG sites

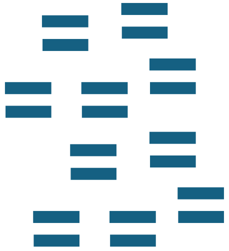


FFPE

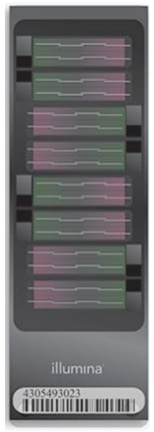
DNA extraction

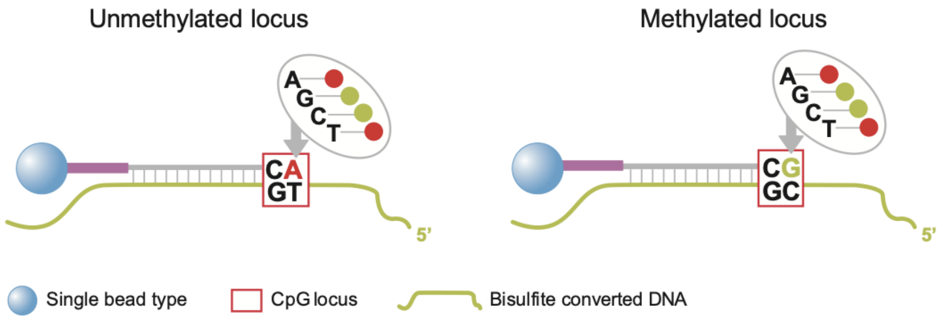
Bisulfite conversion

Genome amplification



Fragmentation

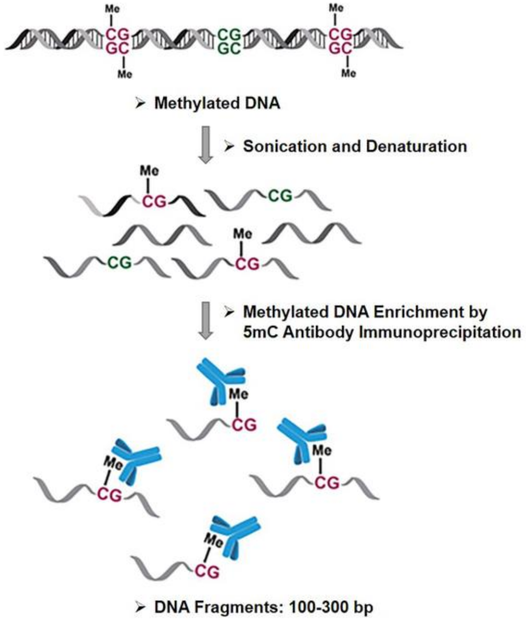




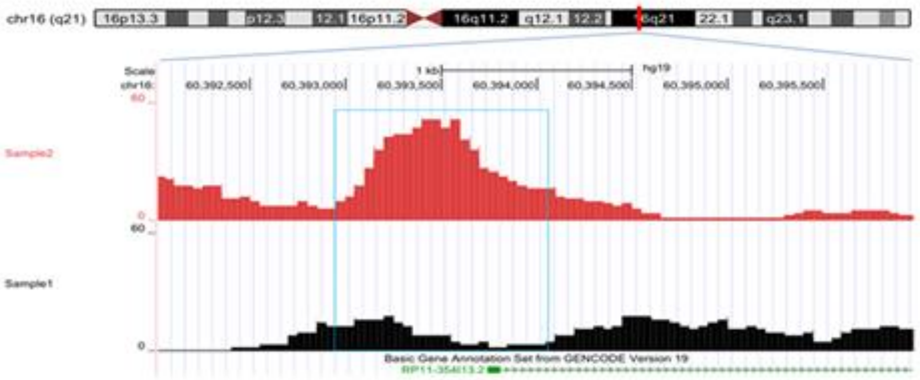
# Enrichment based sequencing methods

## MeDIP-seq, MDB-Seq

- Use antibodies or proteins to bind to methylated DNA
- Lower resolution

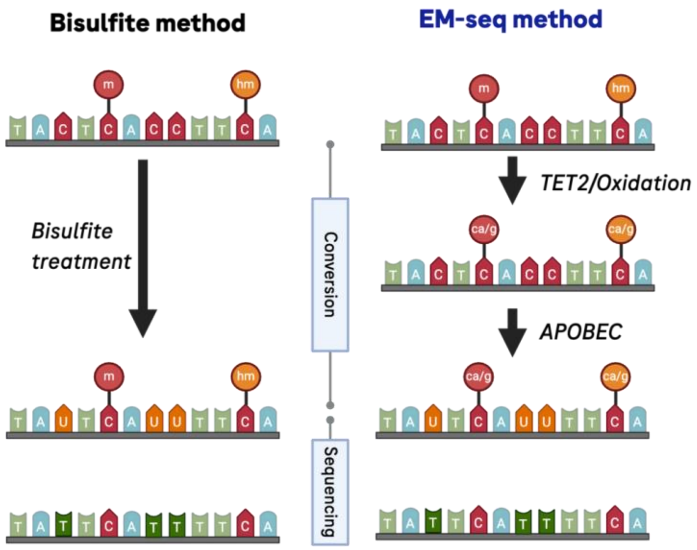


MeDIP seq

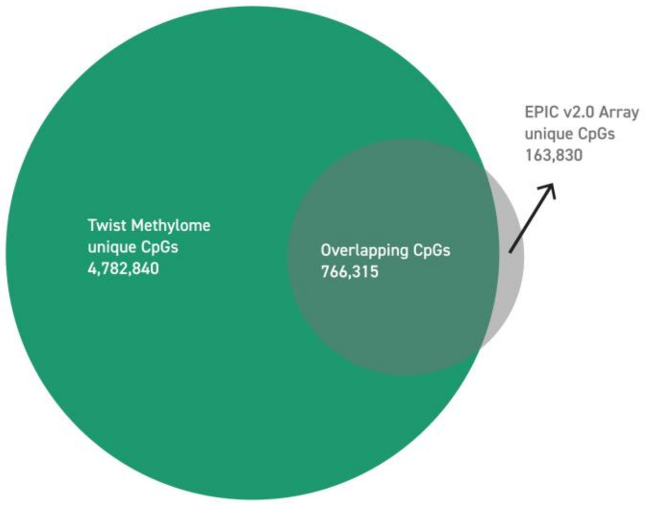


# Enzymatic methyl seq

- Enzymatic conversion
- Less DNA damage compared to bisulfite



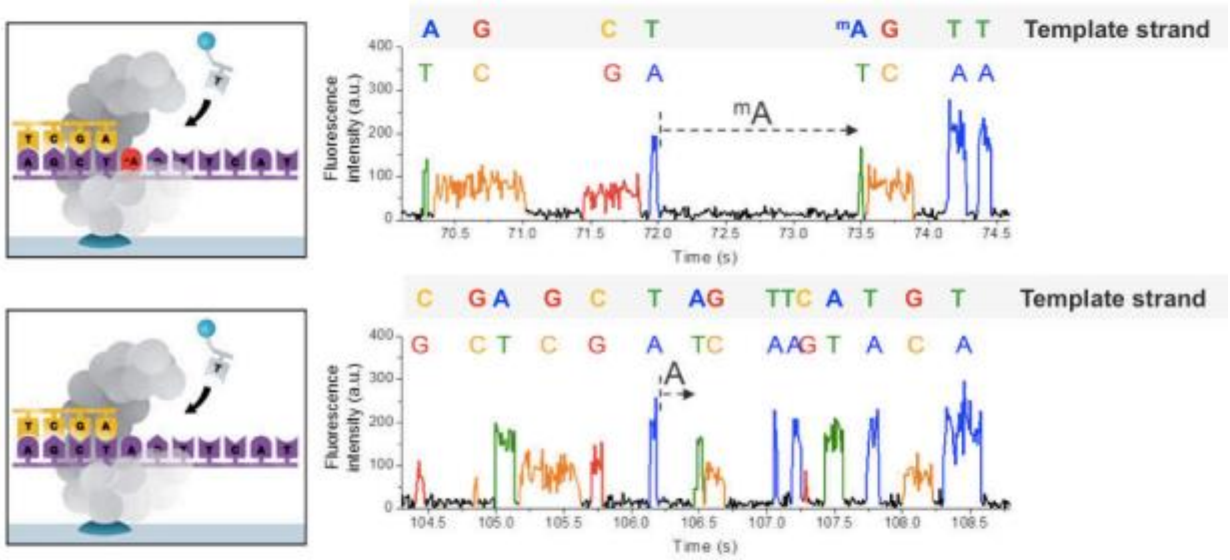
Optionally: combine with targetted panel



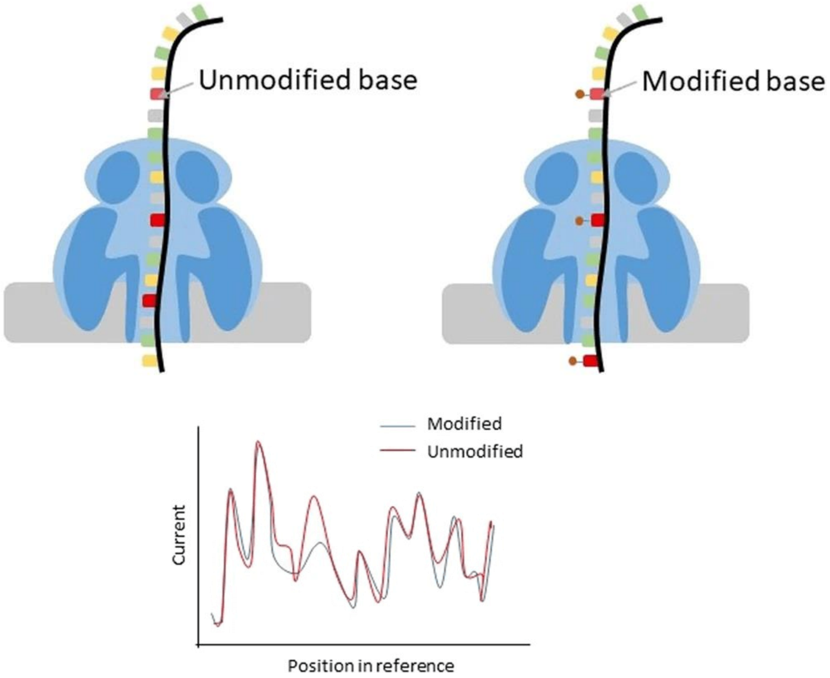
**Human Methylome Panel**

# 3th generation sequencing

- Oxford Nanopore sequencing
- PacBio SMRT Sequencing
- No PCR step! So methylation is not lost!



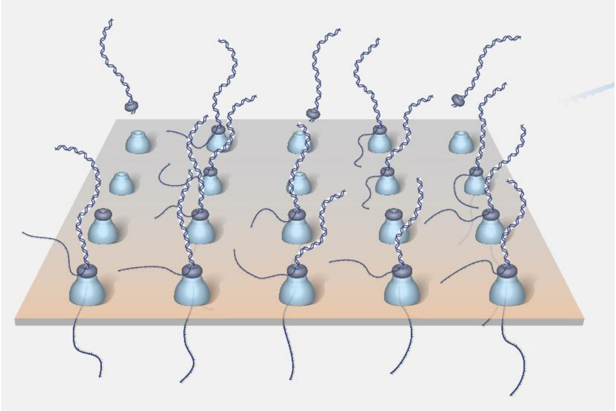
PacBio SMRT seq

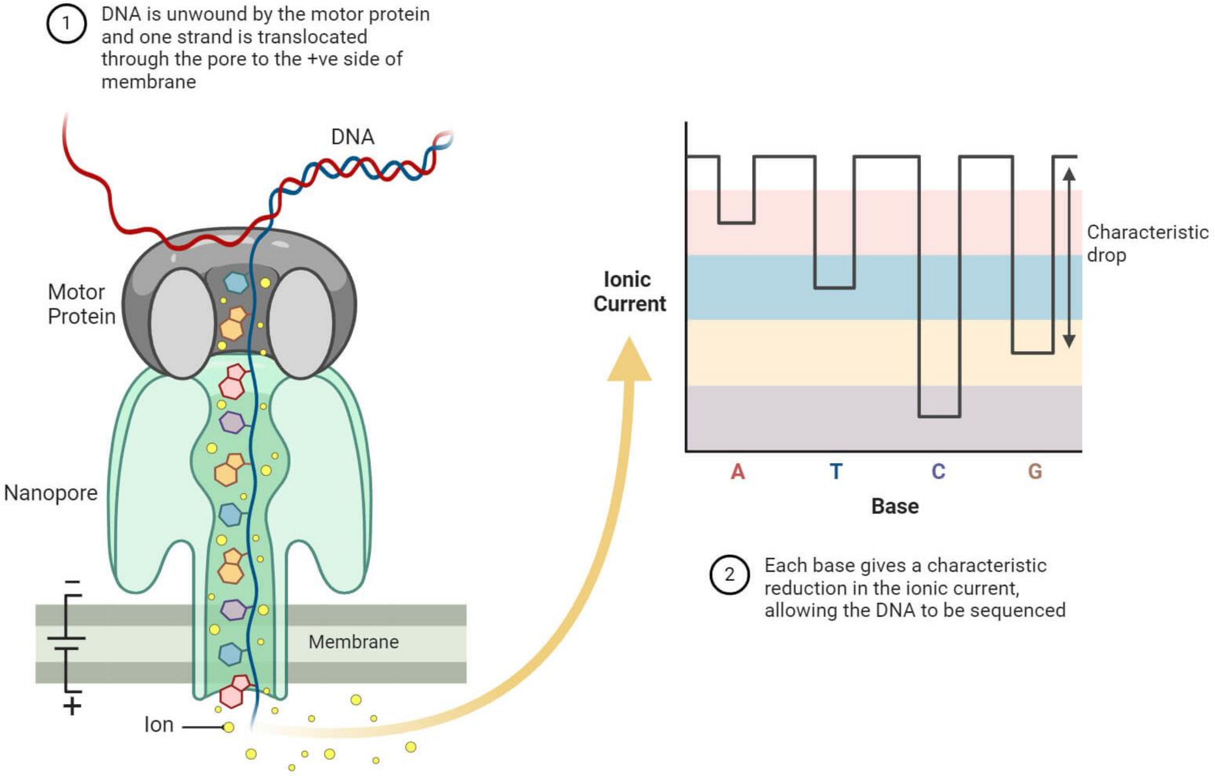


Oxford nanopore

# Oxford Nanopore sequencing technology (ONT)

- Single DNA/RNA molecules pass through nanopores: measure of changes in ionic current
- Long reads (50 bp to >4 Mb)





① DNA is unwound by the motor protein and one strand is translocated through the pore to the +ve side of membrane

② Each base gives a characteristic reduction in the ionic current, allowing the DNA to be sequenced

# ONT advantages

scalability

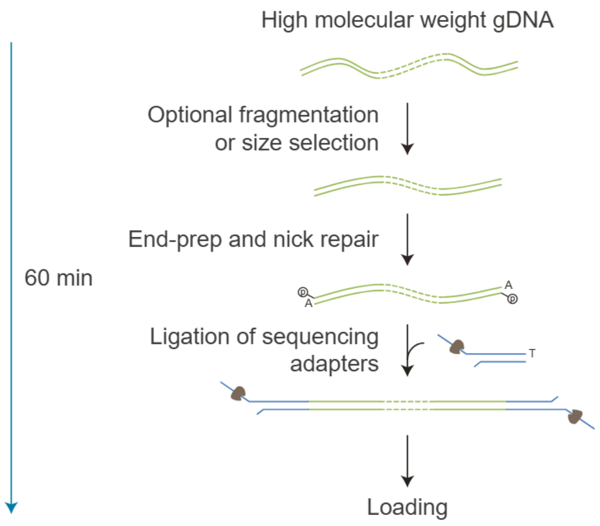
Any fragment length libraries 20 bases to 4 Mb+

Rapid prep in just 10 minutes

PCR-free protocols to eliminate bias

Libraries for whole-genome or targeted approaches

Base modifications preserved



High molecular weight gDNA

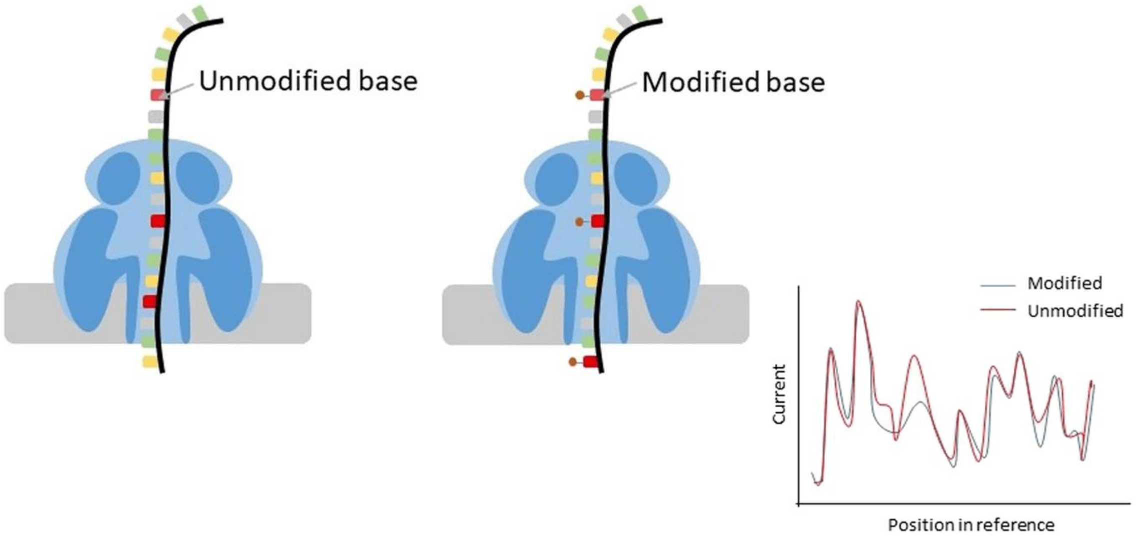
Optional fragmentation or size selection

End-prep and nick repair

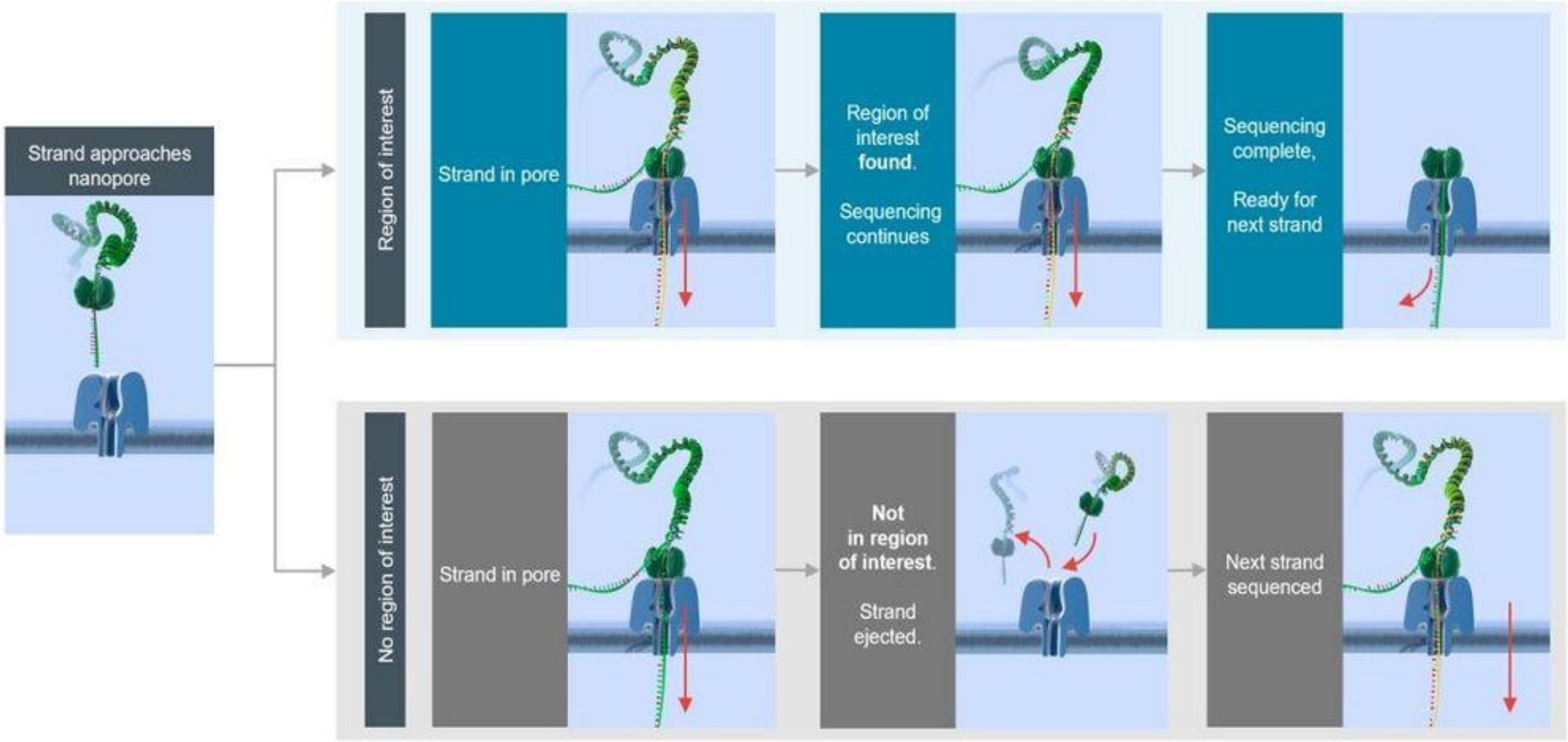
Ligation of sequencing adapters

Loading

60 min



# ONT adaptive sampling



Strand approaches nanopore

Region of interest

Strand in pore

Region of interest **found**.

Sequencing continues

Sequencing complete,

Ready for next strand

No region of interest

Strand in pore

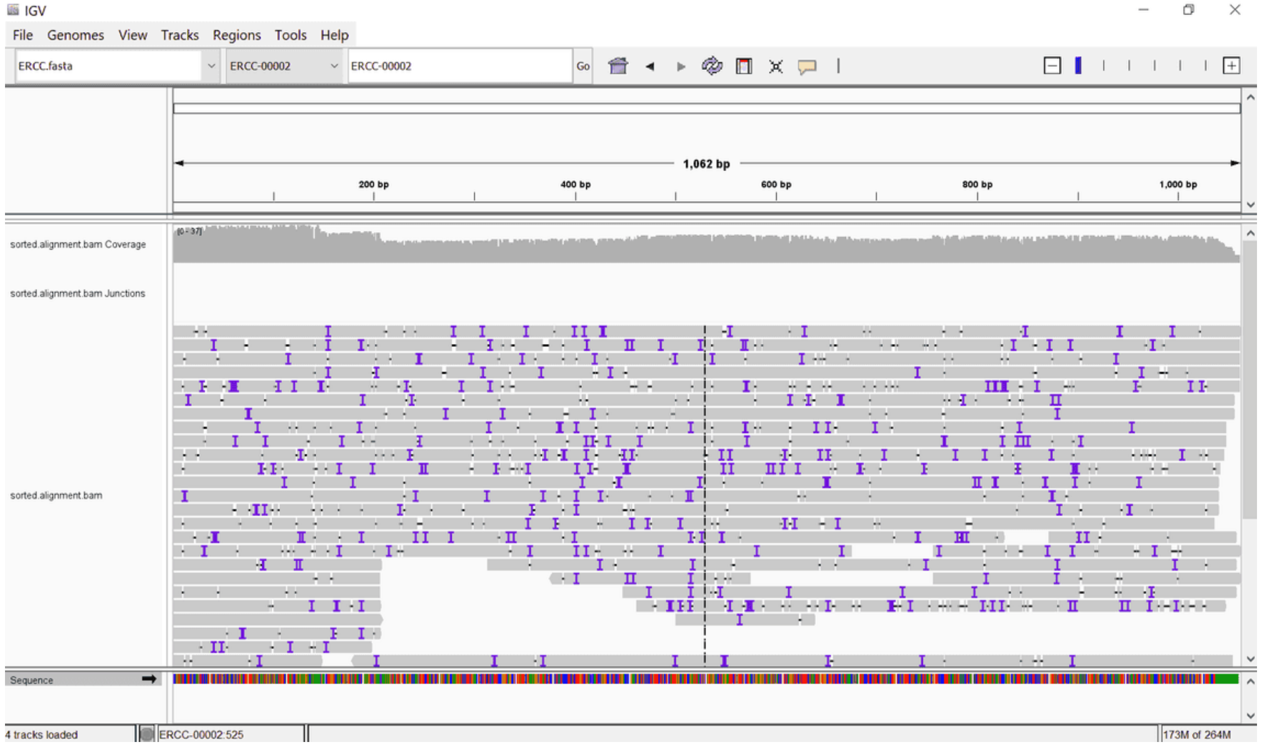
**Not in region of interest.**

Strand ejected.

Next strand sequenced

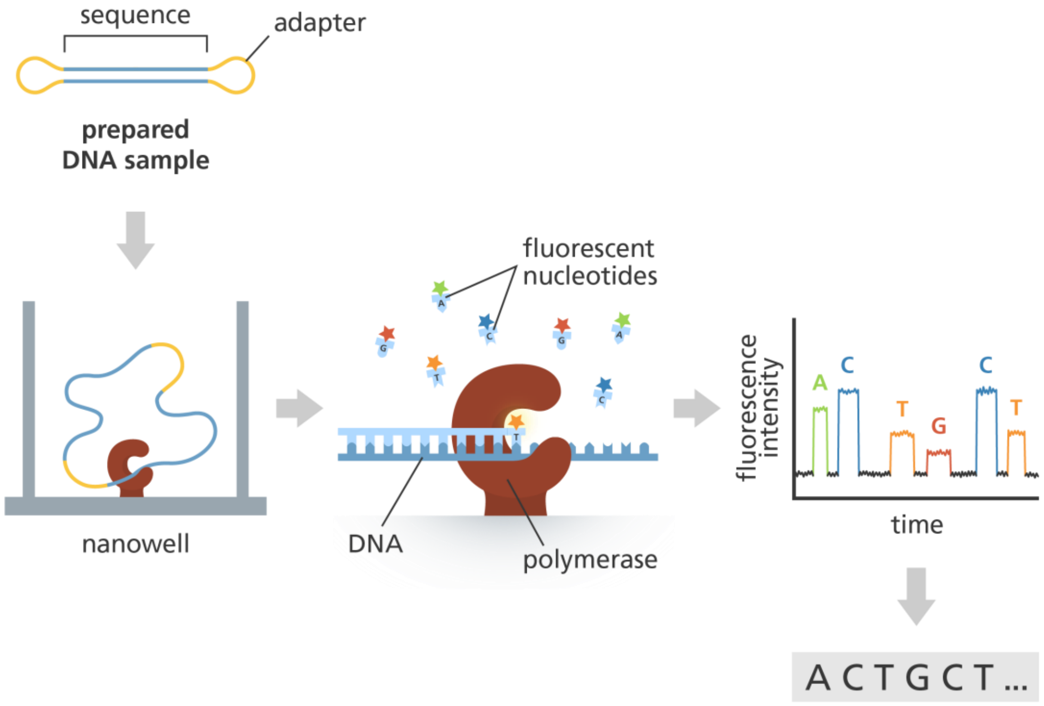
# Disadvantages ONT

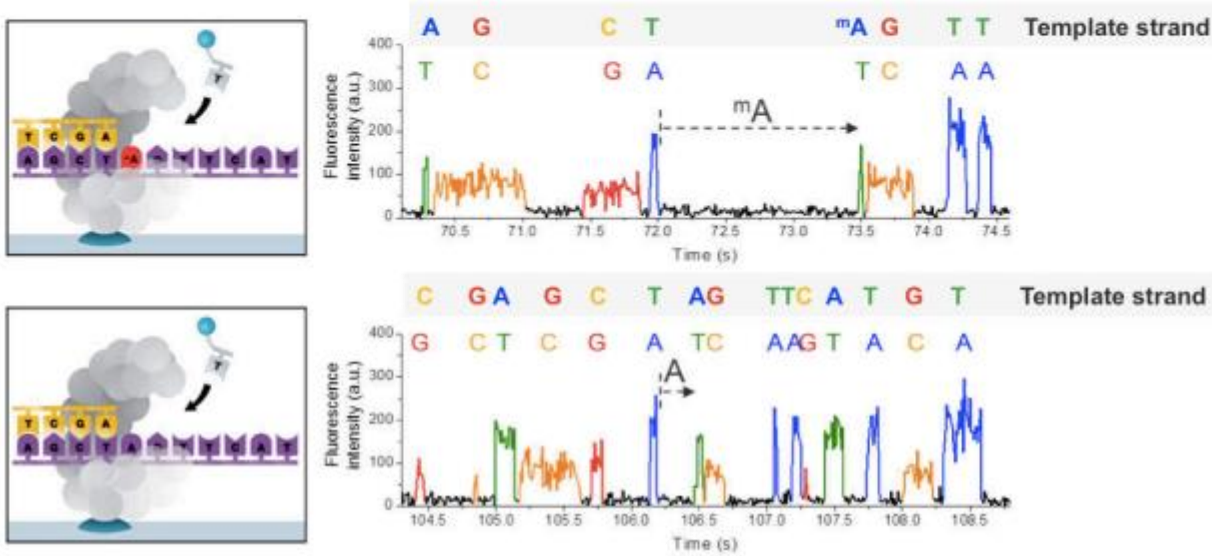
- High error rate, mainly **small insertions/deletions**
- Requires high molecular weight DNA  - difficulties with FFPE matieral
- Higher cost compared to illumina



# PacBio SMRT sequencing

- Long reads
- No PCR, direct modification detection
- HiFi reads are generated by combining information from multiple observations of a single DNA molecule – high accuracy!
- Cost per Gb: Higher than short-read platforms
- **DNA input:** Requires high-quality, high-molecular-weight DNA.





Modification detection!

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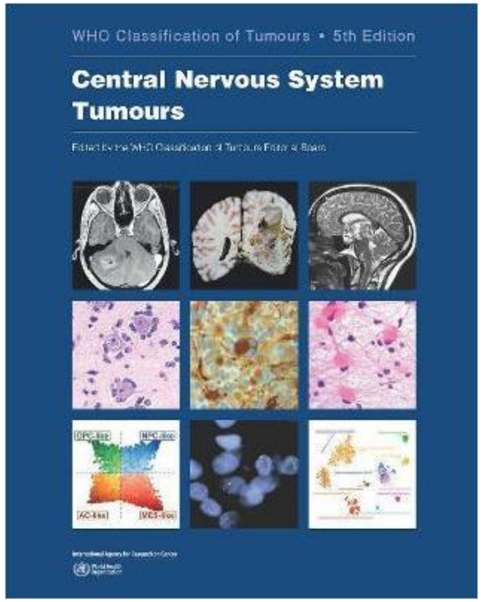
# DNA Methylation as diagnostic tool

- Genome wide methylation profile is determined by
    - Cell of origin
    - Somatically acquired molecular changes
- DNA methylation is highly robust & reproducible even from small samples with poor quality
- Used as finger print for subtyping of brain tumors, sarcoma’s, CUPs,...
- In WHO guideliness for CNS tumors



WHO Classification of Tumours • 5th Edition

**Central Nervous System Tumours**

Louis et al. Neuro Oncol 2021.

# DNA Methylation for CNS tumor classification

>100 known types of central neurvous system tumors

- ranging from benign to highly malignant
- inter observer variablitity based on histology

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>100 known types of central neurvous system tumors

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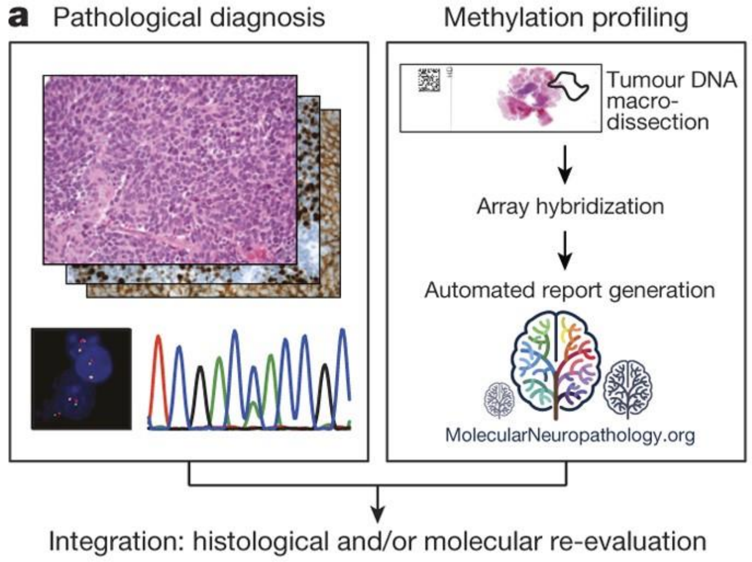
Generation of methylation based classifier based on infinium methylation 450K array

Article | Published: 14 March 2018

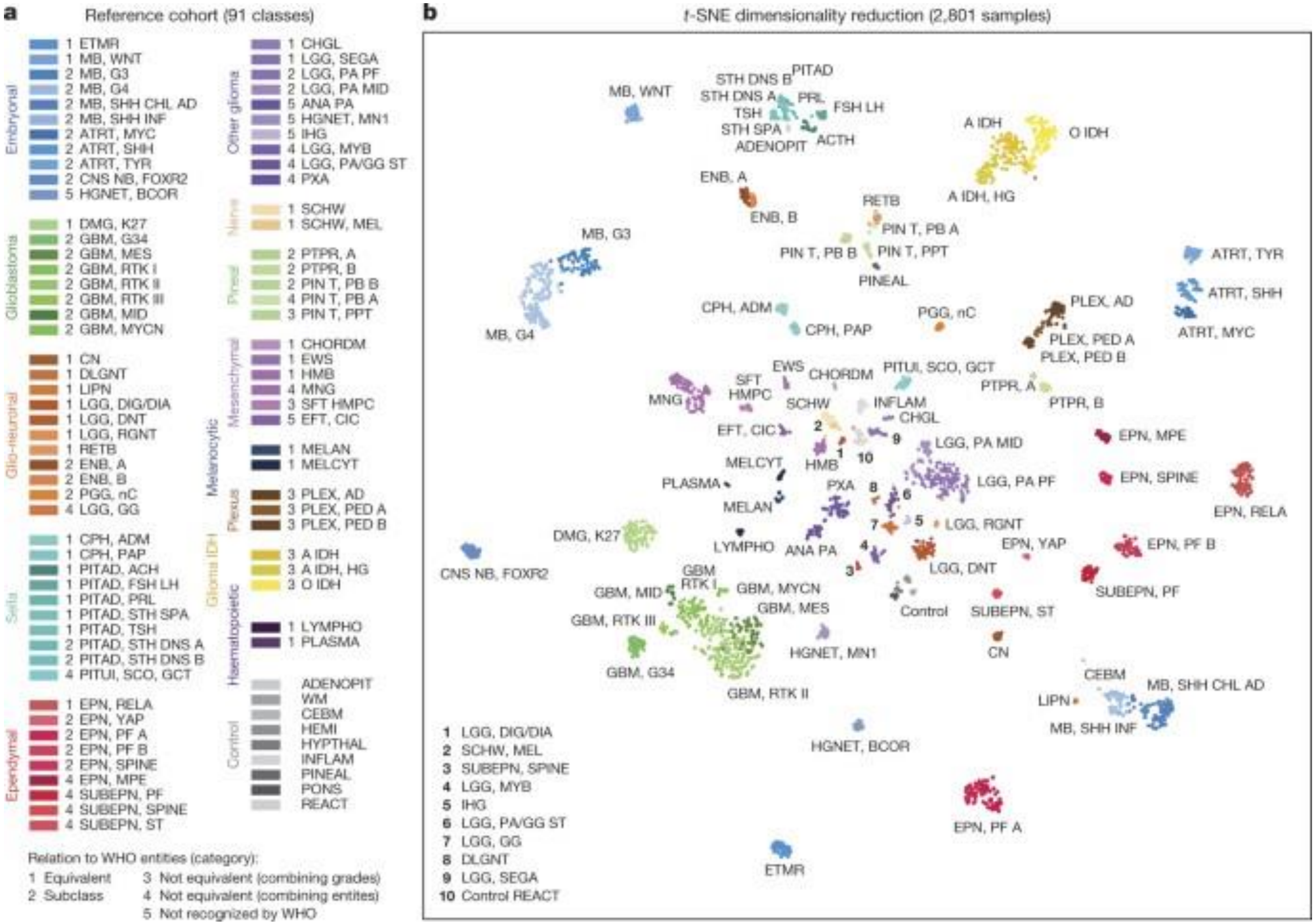
## DNA methylation-based classification of central nervous system tumours

David Capper, David T. W. Jones, Martin Sill, Volker Hovestadt, Daniel Schrimpf, Dominik Sturm, Christian Koelsche, Felix Sahm, Lukas Chavez, David E. Reuss, Annekathrin Kratz, Annika K. Wefers, Kristin Huang, Kristian W. Pajtler, Leonille Schweizer, Damian Stichel, Adriana Olar, Nils W. Engel, Kerstin Lindenberg, Patrick N. Harter, Anne K. Braczynski, Karl H. Plate, Hildegard Dohmen, Boyan K. Garvalov, … Stefan M. Pfister ✉ + Show authors

MNP v11 classifier



*Capper et al. 2018*

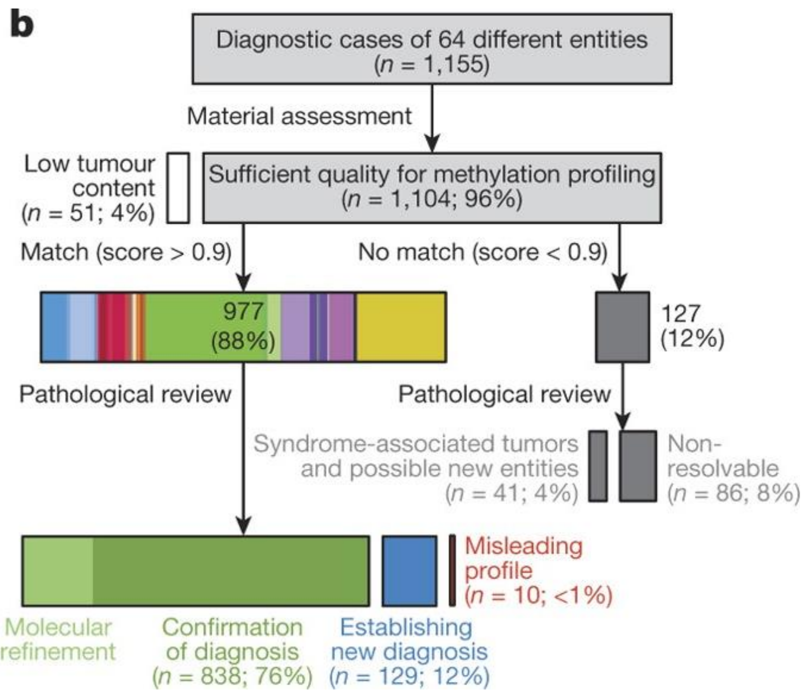


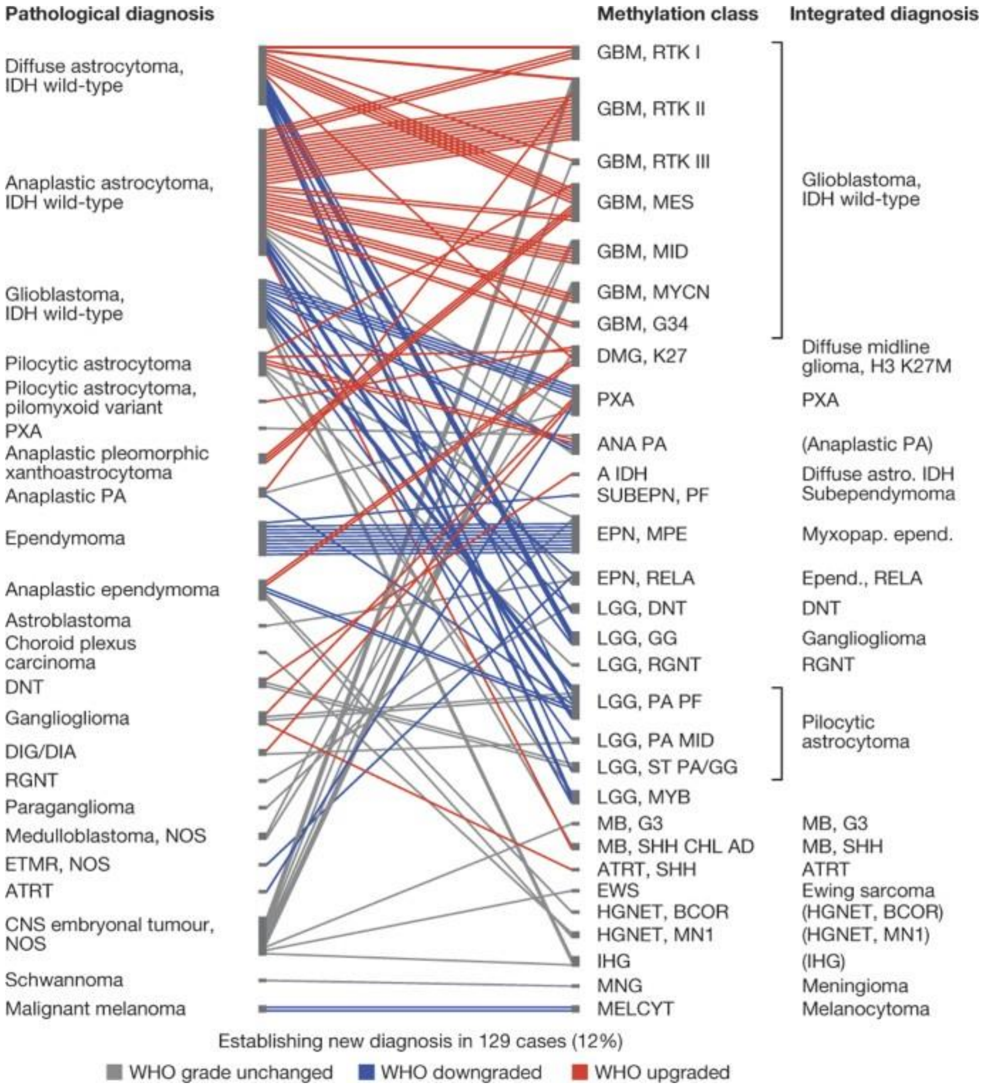
- 2801 samples
- 91 classes (82 CNS tumor classes + micro environment + non malignant CNS regions)

*Capper et al. 2018*

# Prospective validation of the MNP v11 classifier

- 1155 diagnostic CNS tumors
  - 51 (4%) not suited for methylation due to low tumor content
  - 977/1104 (88%) resulted in a classification score >0.9
  - 835/1104 (76%) concordant with pathology
  - 139 non concordant
    - 129 revised (with change grading in 71%)
    - 10 cases discrepant (<1%)



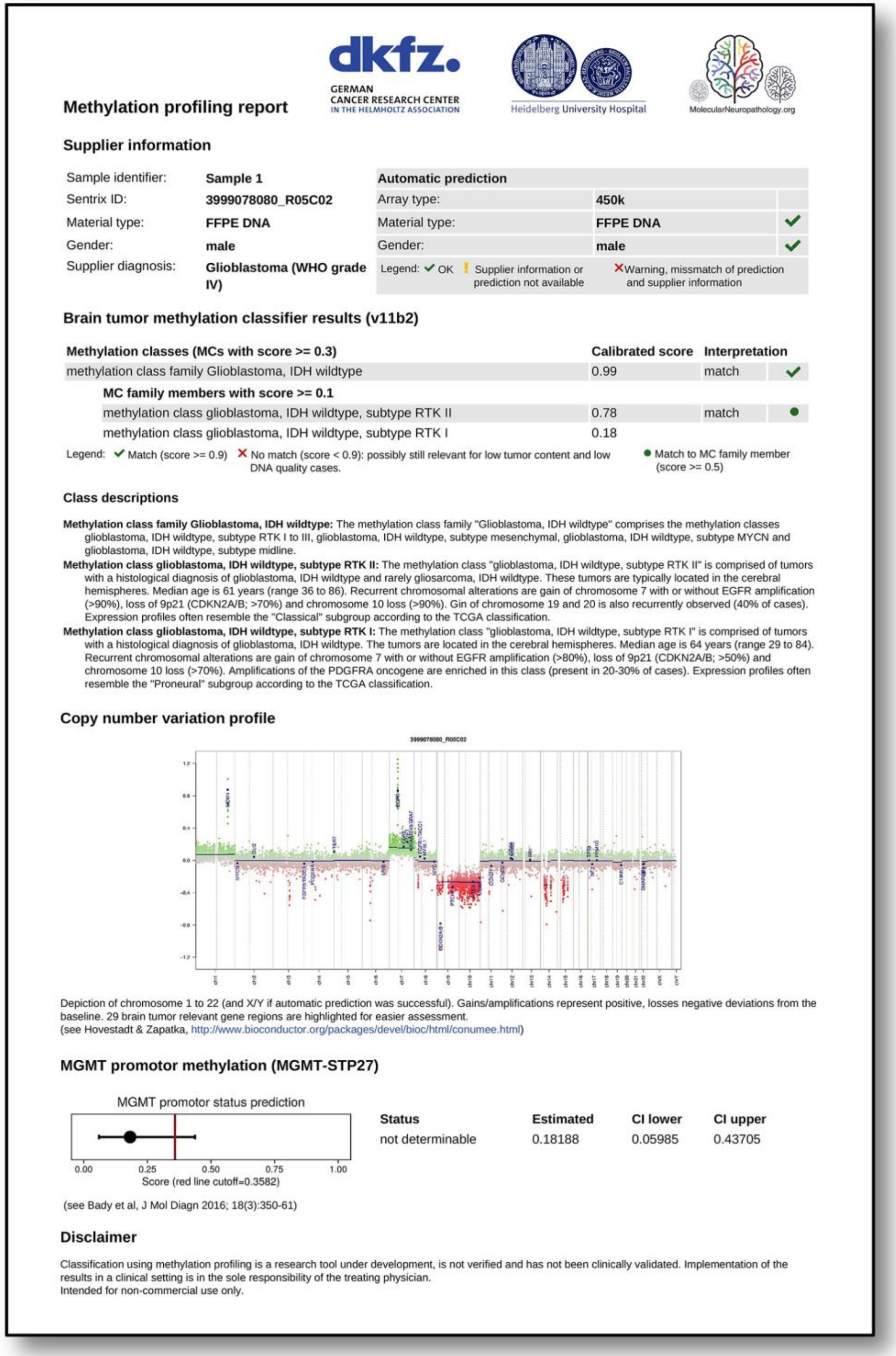


*Capper et al. 2018*

| **Timeline**<br>working days (total days)<br>day of week | **Process** | **Comments** |
|---|---|---|
| Day 1 (1)<br>Tuesday | Material assessment | Methylation profiling requires high tumour content (>70% tumor cells of total cells)Hematoxylin and eosin staining for identification of optimal area. Scraping of tumour cell rich areas from unstained slides (10 slides of 10 µm each) or punch extraction from paraffin block (either 1.5 mm or 3 mm diameter). Cases with low tumor content are avoided when possible. |
| Day 2 (2)<br>Wednesday | DNA extraction (1/2) | Day one of automated DNA extraction (Maxwell 16 FFPE plus LEV DNA purification kit, AS1135, Promega). |
| Day 3 (3)<br>Thursday | DNA extraction (2/2)<br>DNA quality control<br>Bisulfite conversion (1/2) | Day two of DNA extraction.<br>Concentration measurement using Qubit (Qubit dsDNA BR assay kit, Q32853, Invitrogen).<br>Illumina FFPE QC kit only used optionally (e.g. for highly necrotic cases).<br>Day one of bisulfite conversion of 250ng (in exceptional cases as little as 50-100ng) (Zymo EZ DNA methylation kit; D5002, Zymo). |
| Day 4 (4)<br>Friday | Bisulfite conversion (2/2)<br>DNA Restoration | Day two of bisulfite conversion.<br>FFPE DNA Restore (Illumina).<br>DNA cleanup. Part of methylation array kit provided by Illumina. Performed strictly according to instruction manual.<br>Freezing over weekend. |
| Day 5 (7)<br>Monday | Whole genome amplification | Part of methylation array kit provided by Illumina. Performed strictly according to instruction manual. |
| Day 6 (8)<br>Tuesday | Fragmentation, precipitation<br>Resuspension<br>Array hybridization (1/2) | Part of methylation array kit provided by Illumina. Performed strictly according to instruction manual. |
| Day 7 (9)<br>Wednesday | Array hybridization (2/2)<br>Washing and staining<br>Scanning of array<br>Technical quality control | Part of methylation array kit provided by Illumina. Performed strictly according to instruction manual.<br>Technical quality control using assay internal system controls following the instruction manual. Eliminate bad samples. |
| Day 8 (10)<br>Thursday | Data upload to Webportal<br>Integration with pathological findings<br>Writing of reports | Upload to www.molecularneuropathology.org<br>Gender check. Checking for genotype matches if established. Exclusion of case swapping.<br>Integration of pathological findings with methylation profiling. Possibly reconsideration of histology and possibly additional molecular investigations required. |

https://app.epignostix.com

*Capper et al. 2018*



dkfz.
GERMAN
CANCER RESEARCH CENTER
IN THE HELMHOLTZ ASSOCIATION

Heidelberg University Hospital

MolecularNeuropathology.org

## Methylation profiling report

### Supplier information

| | | | | |
|---|---|---|---|---|
| Sample identifier: | **Sample 1** | **Automatic prediction** | | |
| Sentrix ID: | **3999078080_R05C02** | Array type: | **450k** | |
| Material type: | **FFPE DNA** | Material type: | **FFPE DNA** | ✓ |
| Gender: | **male** | Gender: | **male** | ✓ |
| Supplier diagnosis: | **Glioblastoma (WHO grade IV)** | Legend: ✓ OK ! Supplier information or prediction not available | ✗ Warning, missmatch of prediction and supplier information | |

### Brain tumor methylation classifier results (v11b2)

| **Methylation classes (MCs with score >= 0.3)** | **Calibrated score** | **Interpretation** | |
|---|---|---|---|
| methylation class family Glioblastoma, IDH wildtype | 0.99 | match | ✓ |
| **MC family members with score >= 0.1** | | | |
| methylation class glioblastoma, IDH wildtype, subtype RTK II | 0.78 | match | ● |
| methylation class glioblastoma, IDH wildtype, subtype RTK I | 0.18 | | |

Legend: ✓ Match (score >= 0.9) ✗ No match (score < 0.9): possibly still relevant for low tumor content and low DNA quality cases. ● Match to MC family member (score >= 0.5)

### Class descriptions

**Methylation class family Glioblastoma, IDH wildtype:** The methylation class family "Glioblastoma, IDH wildtype" comprises the methylation classes glioblastoma, IDH wildtype, subtype RTK I to III, glioblastoma, IDH wildtype, subtype mesenchymal, glioblastoma, IDH wildtype, subtype MYCN and glioblastoma, IDH wildtype, subtype midline.

**Methylation class glioblastoma, IDH wildtype, subtype RTK II:** The methylation class "glioblastoma, IDH wildtype, subtype RTK II" is comprised of tumors with a histological diagnosis of glioblastoma, IDH wildtype and rarely gliosarcoma, IDH wildtype. These tumors are typically located in the cerebral hemispheres. Median age is 61 years (range 36 to 86). Recurrent chromosomal alterations are gain of chromosome 7 with or without EGFR amplification (>90%), loss of 9p21 (CDKN2A/B; >70%) and chromosome 10 loss (>90%). Gin of chromosome 19 and 20 is also recurrently observed (40% of cases). Expression profiles often resemble the "Classical" subgroup according to the TCGA classification.

**Methylation class glioblastoma, IDH wildtype, subtype RTK I:** The methylation class "glioblastoma, IDH wildtype, subtype RTK I" is comprised of tumors with a histological diagnosis of glioblastoma, IDH wildtype. The tumors are located in the cerebral hemispheres. Median age is 64 years (range 29 to 84). Recurrent chromosomal alterations are gain of chromosome 7 with or without EGFR amplification (>80%), loss of 9p21 (CDKN2A/B; >50%) and chromosome 10 loss (>70%). Amplifications of the PDGFRA oncogene are enriched in this class (present in 20-30% of cases). Expression profiles often resemble the "Proneural" subgroup according to the TCGA classification.

### Copy number variation profile

Depiction of chromosome 1 to 22 (and X/Y if automatic prediction was successful). Gains/amplifications represent positive, losses negative deviations from the baseline. 29 brain tumor relevant gene regions are highlighted for easier assessment.
(see Hovestadt & Zapatka, http://www.bioconductor.org/packages/devel/bioc/html/conumee.html)

### MGMT promotor methylation (MGMT-STP27)

| Status | Estimated | CI lower | CI upper |
|---|---|---|---|
| not determinable | 0.18188 | 0.05985 | 0.43705 |

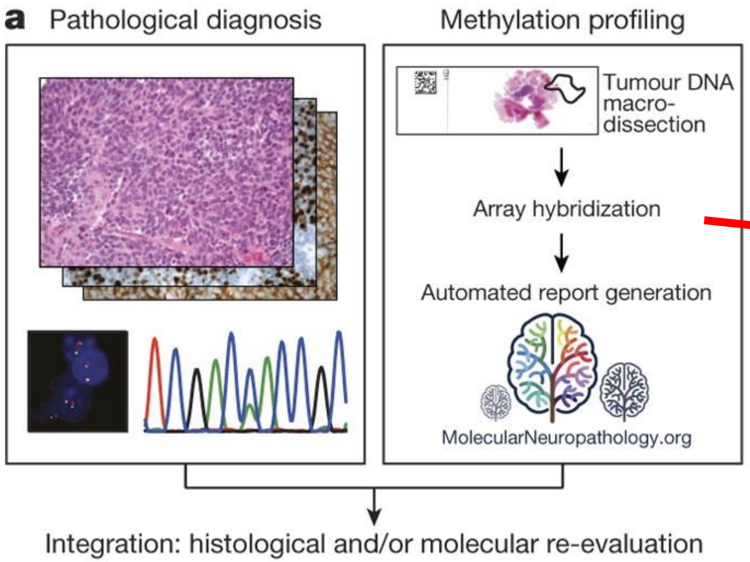
(see Bady et al, J Mol Diagn 2016; 18(3):350-61)

### Disclaimer

Classification using methylation profiling is a research tool under development, is not verified and has not been clinically validated. Implementation of the results in a clinical setting is in the sole responsibility of the treating physician.
Intended for non-commercial use only.

*Capper et al. 2018*

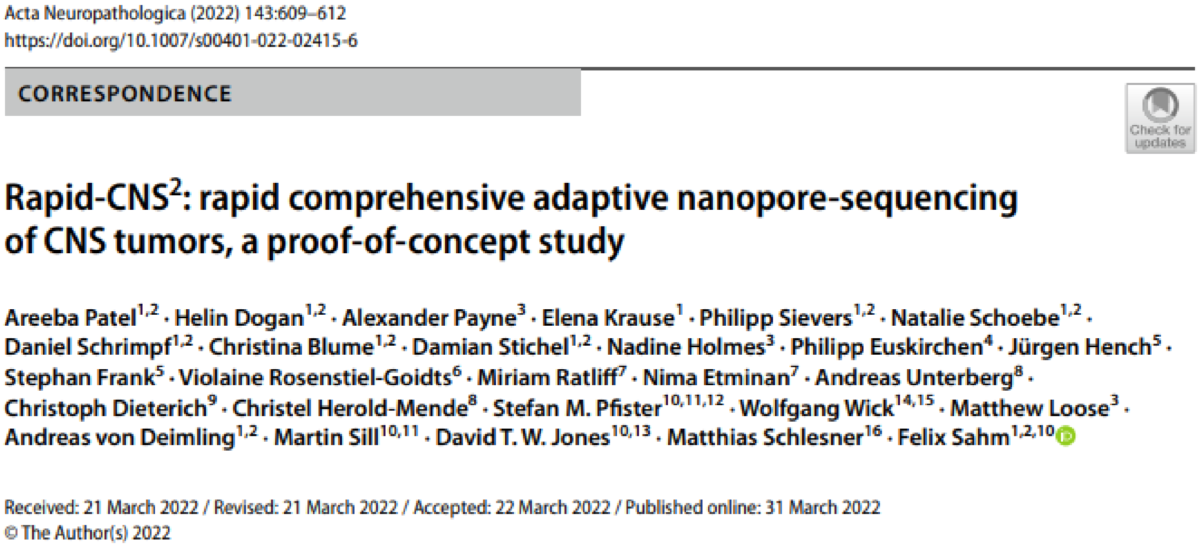
# MNP classifier is array based!



! Disadvantage: still array based!

- Labor intensive
- Minimum of 8 samples needed
- Expensive
- Long turn around time

# Rapid CNS$^2$: a nanopore based classifier



Acta Neuropathologica (2022) 143:609–612
https://doi.org/10.1007/s00401-022-02415-6

CORRESPONDENCE

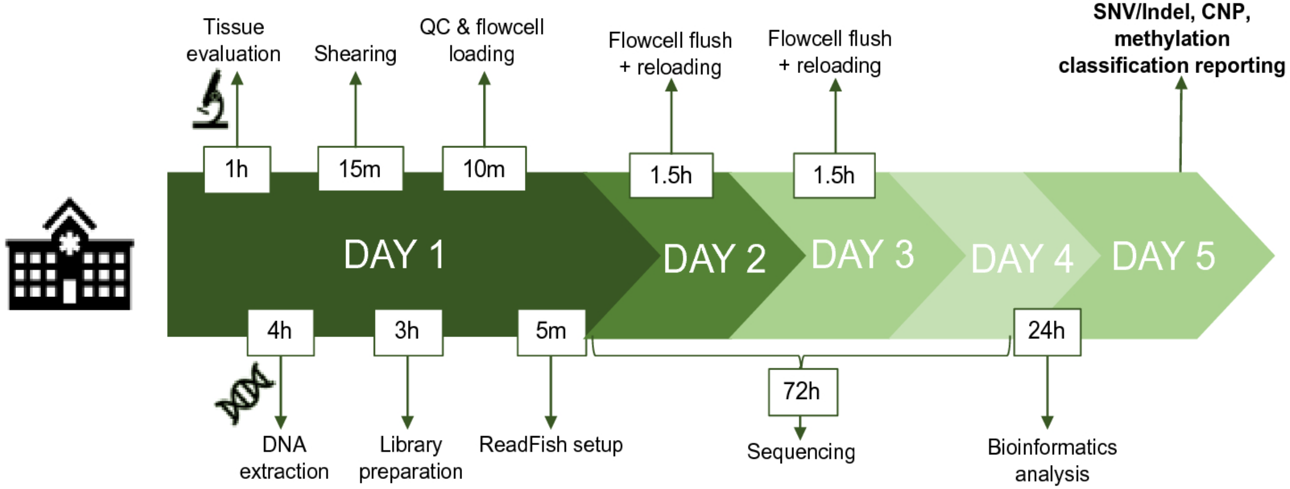
## Rapid-CNS$^2$: rapid comprehensive adaptive nanopore-sequencing of CNS tumors, a proof-of-concept study

Areeba Patel[1,2] · Helin Dogan[1,2] · Alexander Payne[3] · Elena Krause[1] · Philipp Sievers[1,2] · Natalie Schoebe[1,2] · Daniel Schrimpf[1,2] · Christina Blume[1,2] · Damian Stichel[1,2] · Nadine Holmes[3] · Philipp Euskirchen[4] · Jürgen Hench[5] · Stephan Frank[5] · Violaine Rosenstiel-Goidts[6] · Miriam Ratliff[7] · Nima Etminan[7] · Andreas Unterberg[8] · Christoph Dieterich[9] · Christel Herold-Mende[8] · Stefan M. Pfister[10,11,12] · Wolfgang Wick[14,15] · Matthew Loose[3] · Andreas von Deimling[1,2] · Martin Sill[10,11] · David T. W. Jones[10,13] · Matthias Schlesner[16] · Felix Sahm[1,2,10]

Received: 21 March 2022 / Revised: 21 March 2022 / Accepted: 22 March 2022 / Published online: 31 March 2022

Goal:
- Methylation analysis
- Mutation detection
- CNV analysis
- MGMT promoter methylation detection

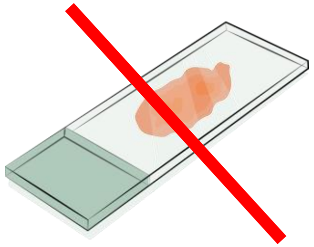
→ Through Nanopore sequencing



Tissue evaluation – 1h
Shearing – 15m
QC & flowcell loading – 10m
Flowcell flush + reloading – 1.5h
Flowcell flush + reloading – 1.5h
**SNV/Indel, CNP, methylation classification reporting**

DAY 1
DAY 2
DAY 3
DAY 4
DAY 5

4h – DNA extraction
3h – Library preparation
5m – ReadFish setup
72h – Sequencing
24h – Bioinformatics analysis

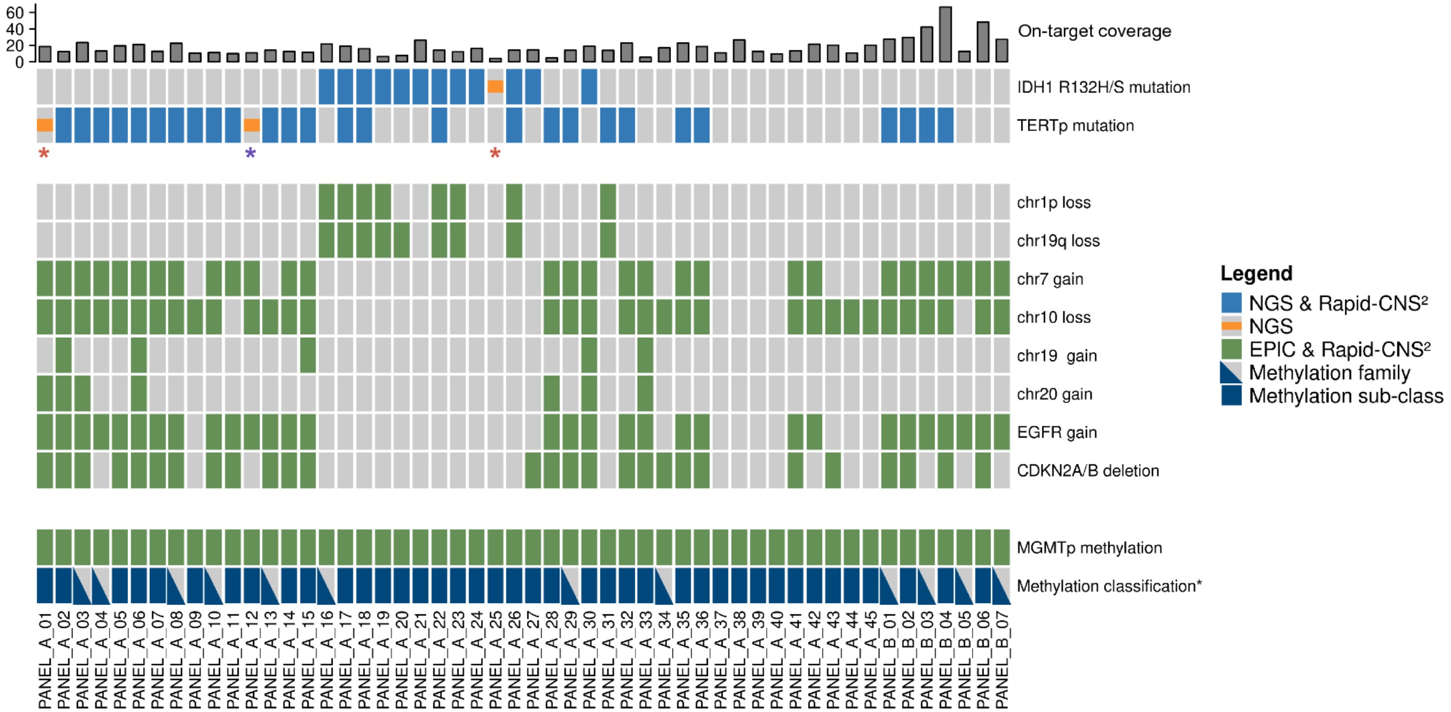


FFPE

*Patel et al. 2022*

# Rapid CNS$^2$: a nanopore based classifier

- Trained on the MNP data v11
- Adaptive sequencing:
    - Epic array sites
    - Brain tumor NGS panel
- 37/45 samples concordant subclass to epic - MNP analysis
- 2 TERTp mutations, 1 IDH1 mutation missed → low tumor content?
- 24h sequencing on MinION sufficient



*Patel et al. 2022*

# Rapid CNS$^2$: a nanopore based classifier

- Trained on the MNP data v11
- Adaptive sequencing:
  - Epic array sites
  - Brain tumor NGS panel
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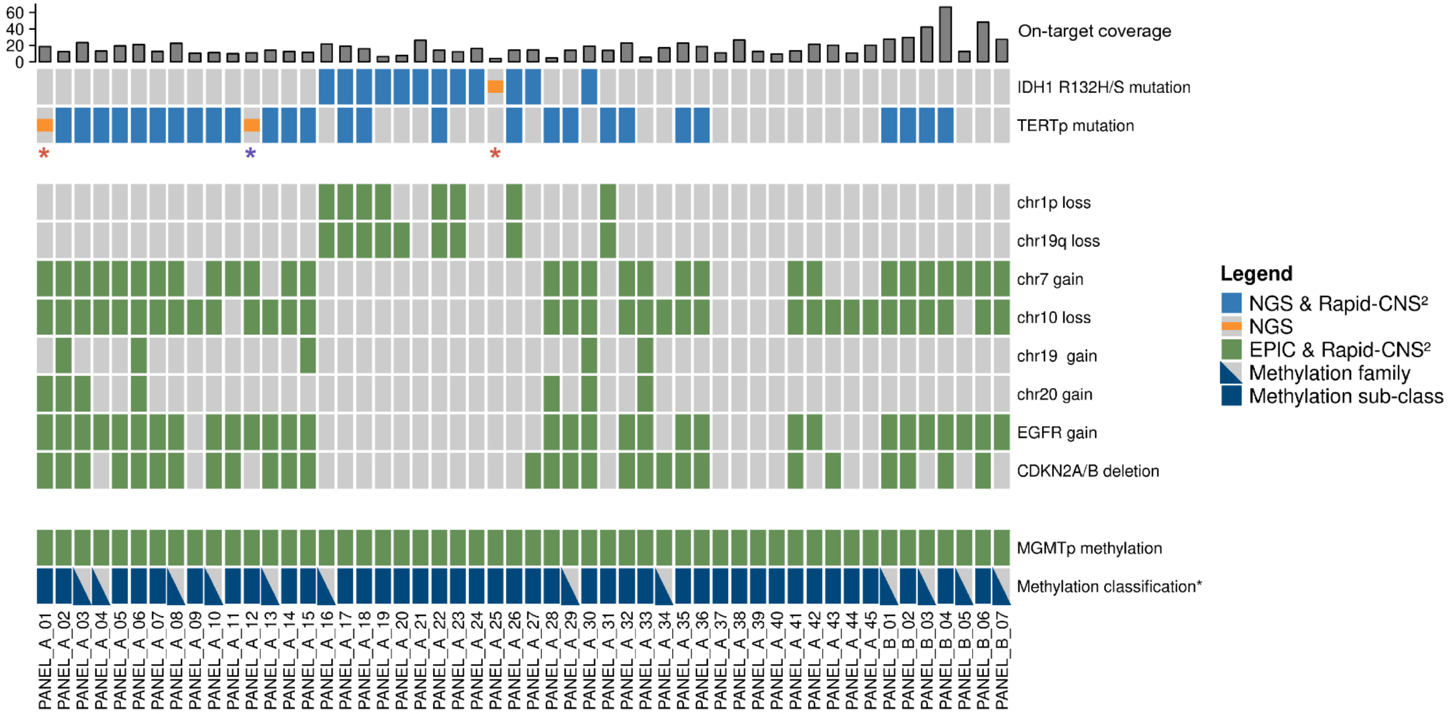
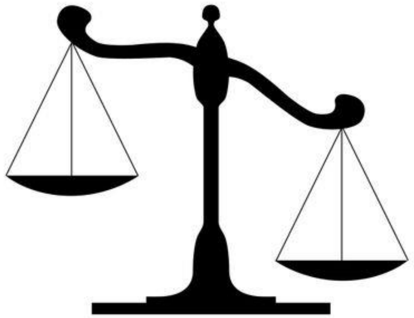
- 2 TERTp mutations, 1 IDH1 mutation missed → low tumor content?
- 24h sequencing on MinION sufficient

Could go faster?



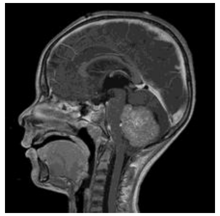
*Patel et al. 2022*

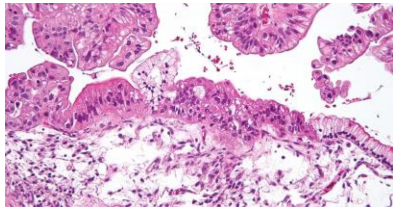
# Sturgeon: classification during surgery

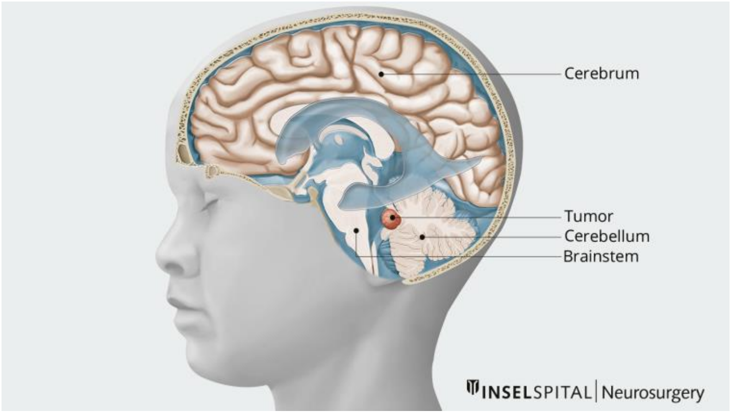


Brain tumor surgery: balance between maximizing tumor resection and minimizing risk of neurological damage and comorbidity

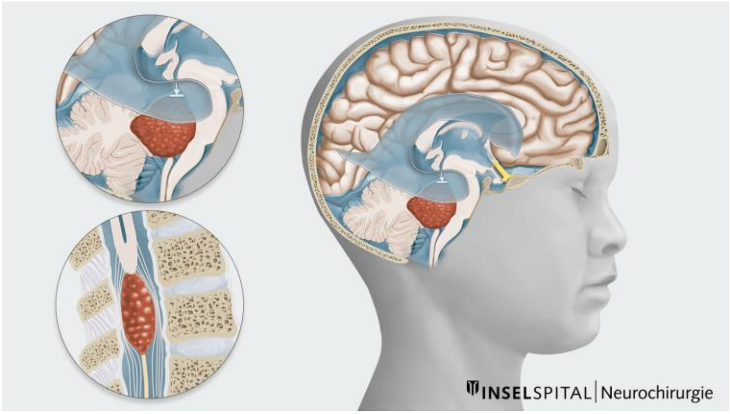
→ Based on pre-operative imaging and intraoperative diagnosis on histology on frozen section







medulloblastoma: limited pronostic improvement between near and total resection



Posterior fossa ependymoma: total resection is very important!

# Sturgeon: classification during surgery

- Nanopore rapid library preps allow very fast library prep
- During surgery: turnaround time of 90 min

Article | Open access | Published: 11 October 2023

**Ultra-fast deep-learned CNS tumour classification during surgery**

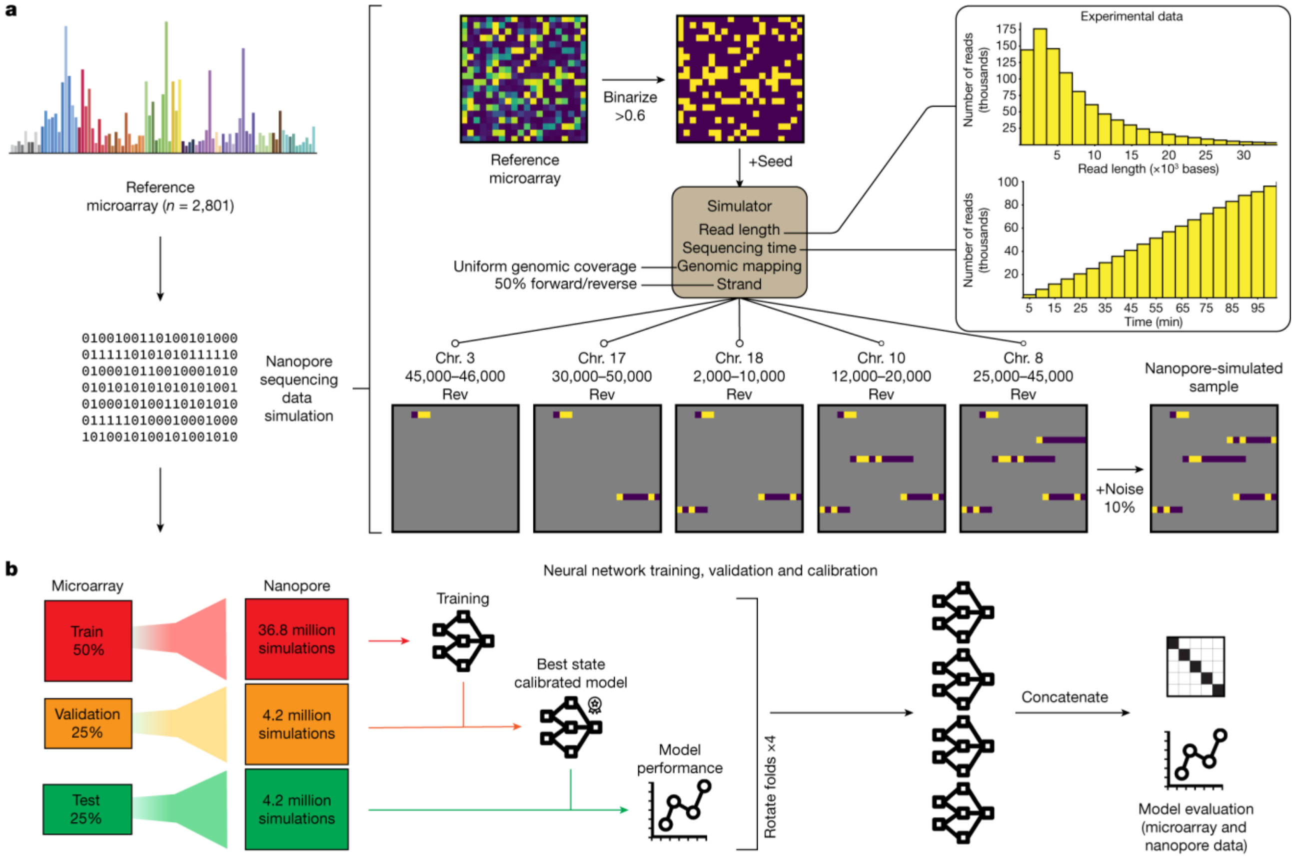
C. Vermeulen, M. Pagès-Gallego, L. Kester, M. E. G. Kranendonk, P. Wesseling, N. Verburg, P. de Witt Hamer, E. J. Kooi, L. Dankmeijer, J. van der Lugt, K. van Baarsen, E. W. Hoving, B. B. J. Tops ✉ & J. de Ridder ✉

*Nature* **622**, 842–849 (2023) | Cite this article

**89k** Accesses | **177** Citations | **580** Altmetric | Metrics

# Building a model on sparse data

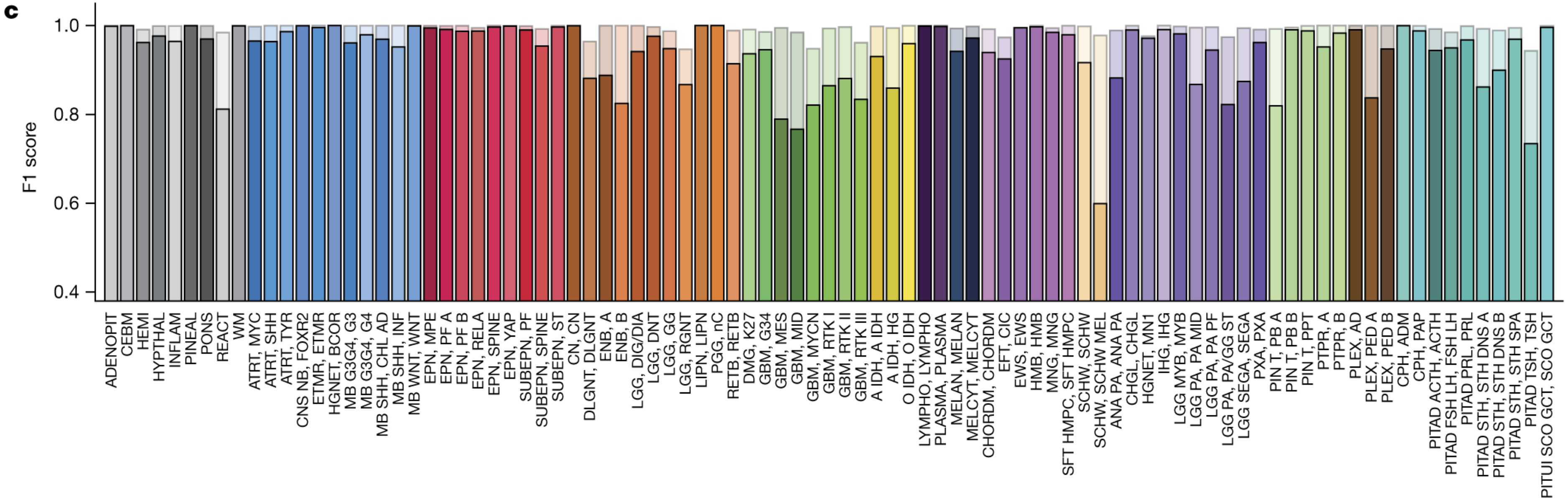
- Classifier specific for **sparse** data!
  → 0.5 – 4% of CpG sites of 450k array, unknown which sites will be covered
- Trained the model on ‘simulated nanopore data’ based on the MNP micro array reference dataset



*Vermeulen et al.,2023*

# Building a model on sparse data

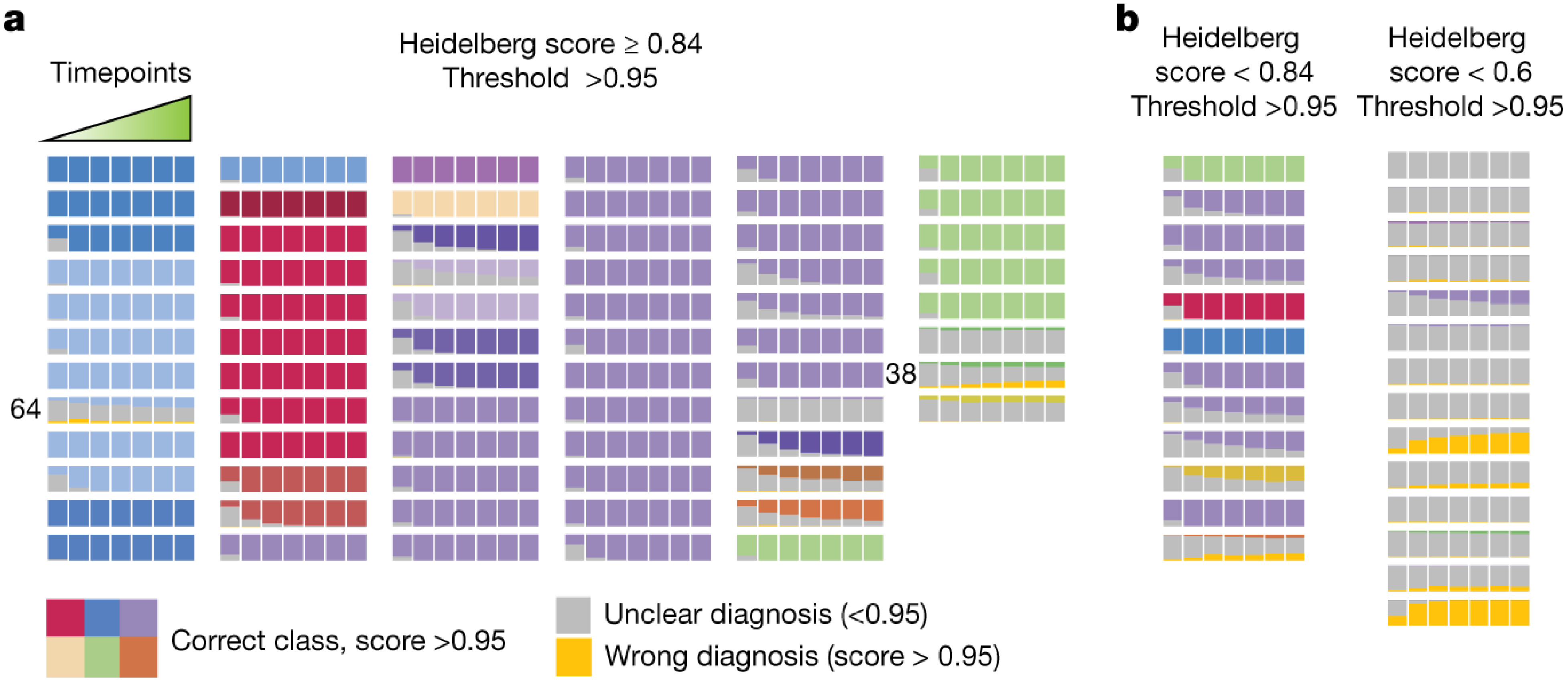
0.935 F1 score across all classes, at 40 min sequencing
0.984 F1 score at familiy level
Low scores are found for subclasses with higher similarity



F1 score = a measure of predictive performance

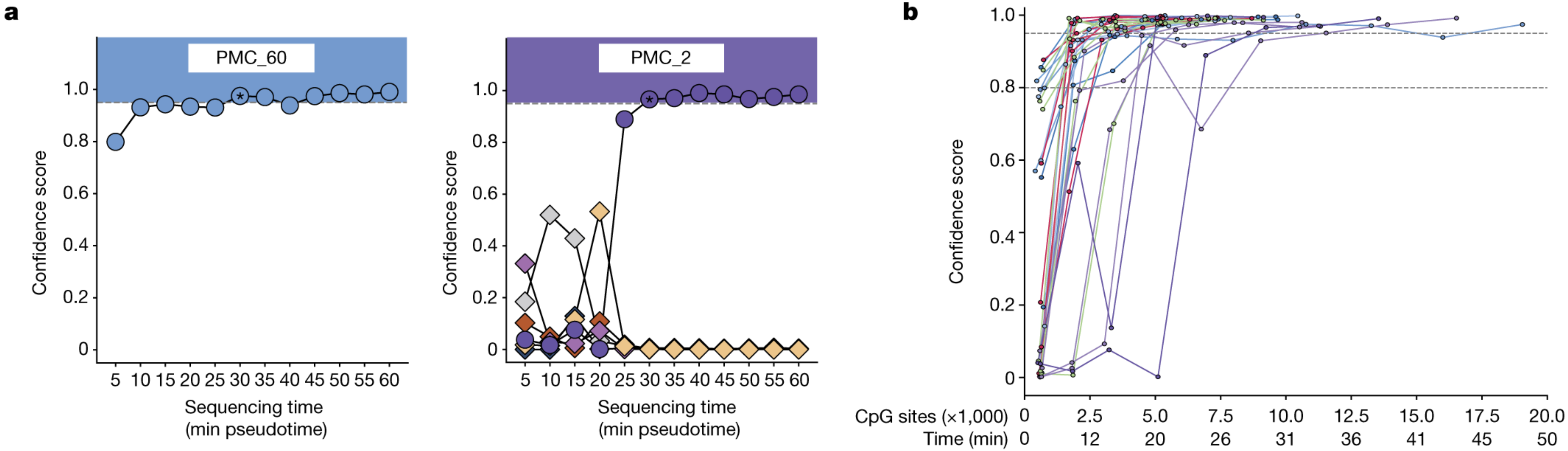
# Testing the model on simulated nanopore runs from array data

- For each array experiment: 500 simulated *sparse* nanopore runs
- Conclusive diagnosis reached within 25-50 min



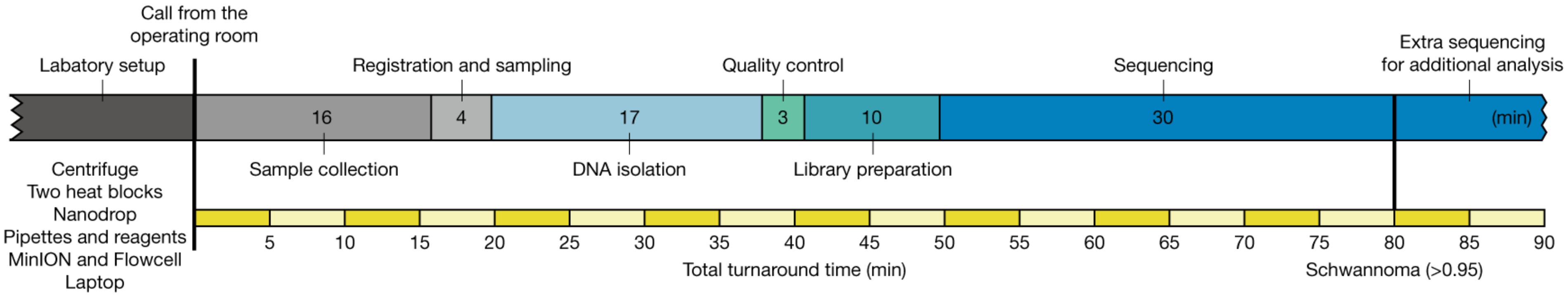
# Testing the model on nanopore data

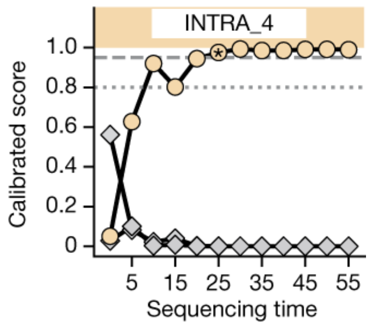
- Correct with high confidence in 77% of samples
- Confidently incorrect in 0.03%
- 25 min minION sequencing



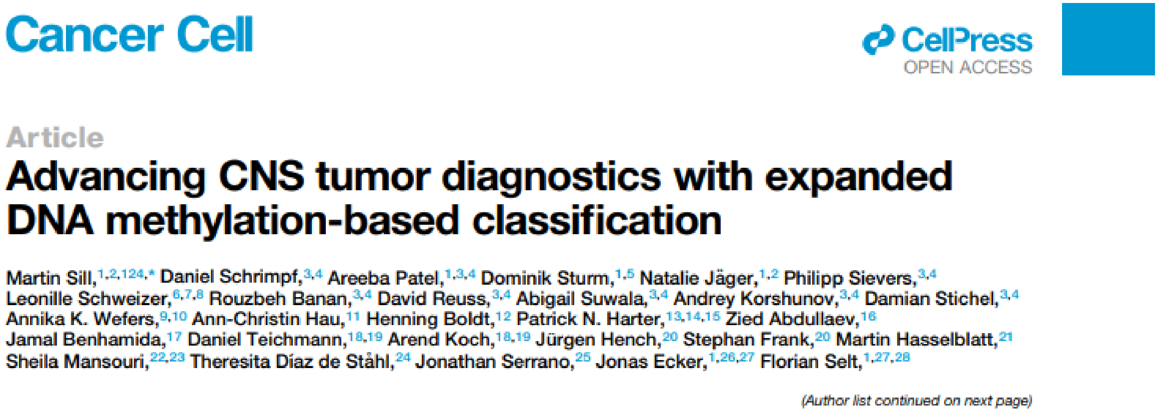
# Sturgeon during surgery

- Correct classification in 72% (18/15) during surgery
- No clear classification for others: not present in reference set, low tumor purity





# Expanded methylation classifier: v12.8



**Cancer Cell**

CellPress OPEN ACCESS

Article

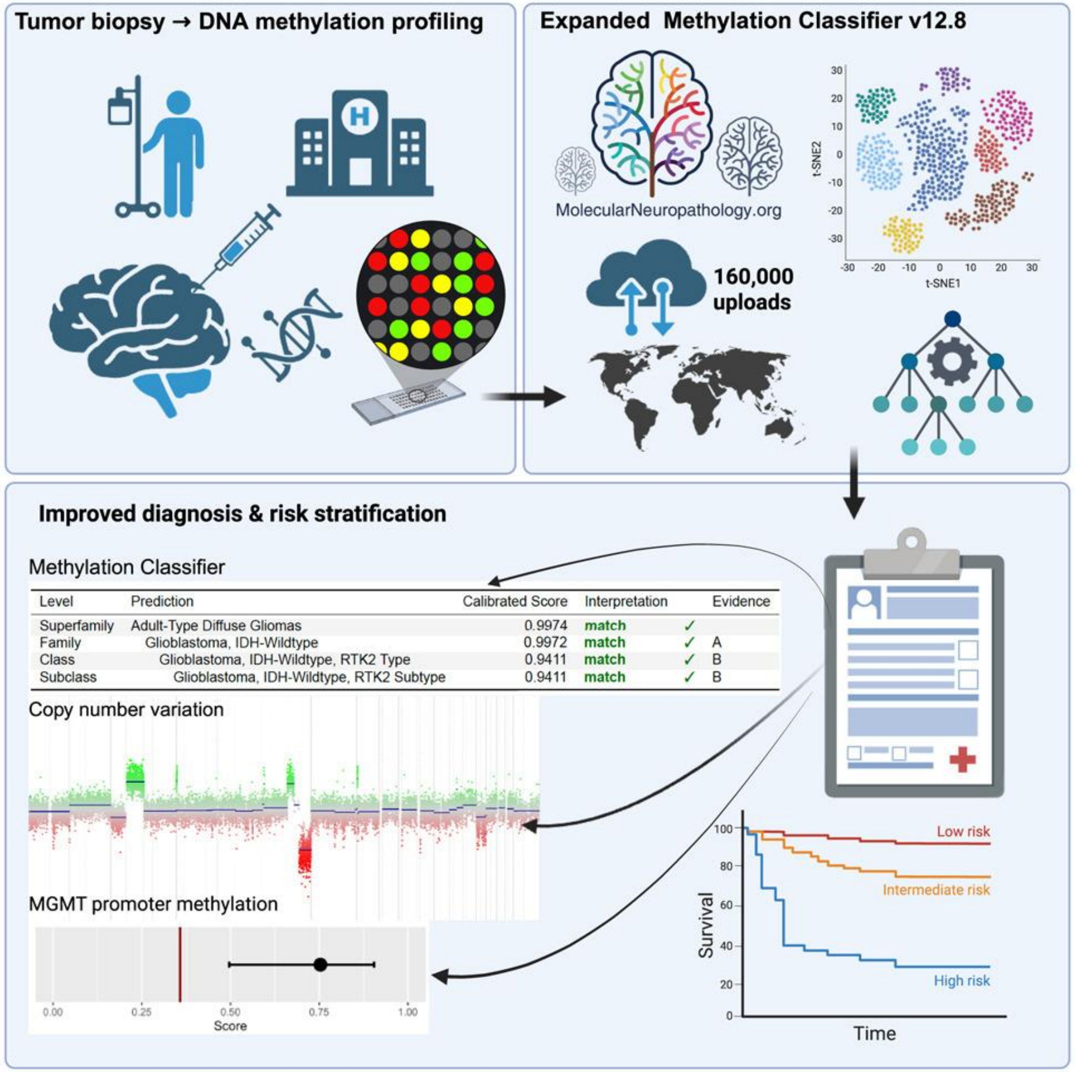
## Advancing CNS tumor diagnostics with expanded DNA methylation-based classification

Martin Sill,[1,2,124,*] Daniel Schrimpf,[3,4] Areeba Patel,[1,3,4] Dominik Sturm,[1,5] Natalie Jäger,[1,2] Philipp Sievers,[3,4] Leonille Schweizer,[6,7,8] Rouzbeh Banan,[3,4] David Reuss,[3,4] Abigail Suwala,[3,4] Andrey Korshunov,[3,4] Damian Stichel,[3,4] Annika K. Wefers,[9,10] Ann-Christin Hau,[11] Henning Boldt,[12] Patrick N. Harter,[13,14,15] Zied Abdullaev,[16] Jamal Benhamida,[17] Daniel Teichmann,[18,19] Arend Koch,[18,19] Jürgen Hench,[20] Stephan Frank,[20] Martin Hasselblatt,[21] Sheila Mansouri,[22,23] Theresita Díaz de Ståhl,[24] Jonathan Serrano,[25] Jonas Ecker,[1,26,27] Florian Selt,[1,27,28]

*(Author list continued on next page)*

2018 --> 2025: many EPIC data collected from all around the world

-7495 CNS profiles
- **184 subclasses**
- Four-tier system
    - Superfamily
    - Family
    - Class
    - subclass



| Level | Prediction | Calibrated Score | Interpretation | | Evidence |
|---|---|---|---|---|---|
| Superfamily | Adult-Type Diffuse Gliomas | 0.9974 | match | ✓ | |
| Family | Glioblastoma, IDH-Wildtype | 0.9972 | match | ✓ | A |
| Class | Glioblastoma, IDH-Wildtype, RTK2 Type | 0.9411 | match | ✓ | B |
| Subclass | Glioblastoma, IDH-Wildtype, RTK2 Subtype | 0.9411 | match | ✓ | B |

*Sill et al. 2025*

# Expanded methylation classifier: v12.8

Cancer Cell

CellPress OPEN ACCESS

Article

## Advancing CNS tumor diagnostics with expanded DNA methylation-based classification

Martin Sill,[1,2,124,*] Daniel Schrimpf,[3,4] Areeba Patel,[1,3,4] Dominik Sturm,[1,5] Natalie Jäger,[1,2] Philipp Sievers,[3,4] Leonille Schweizer,[6,7,8] Rouzbeh Banan,[3,4] David Reuss,[3,4] Abigail Suwala,[3,4] Andrey Korshunov,[3,4] Damian Stichel,[3,4] Annika K. Wefers,[9,10] Ann-Christin Hau,[11] Henning Boldt,[12] Patrick N. Harter,[13,14,15] Zied Abdullaev,[16] Jamal Benhamida,[17] Daniel Teichmann,[18,19] Arend Koch,[18,19] Jürgen Hench,[20] Stephan Frank,[20] Martin Hasselblatt,[21] Sheila Mansouri,[22,23] Theresita Díaz de Ståhl,[24] Jonathan Serrano,[25] Jonas Ecker,[1,26,27] Florian Selt,[1,27,28]

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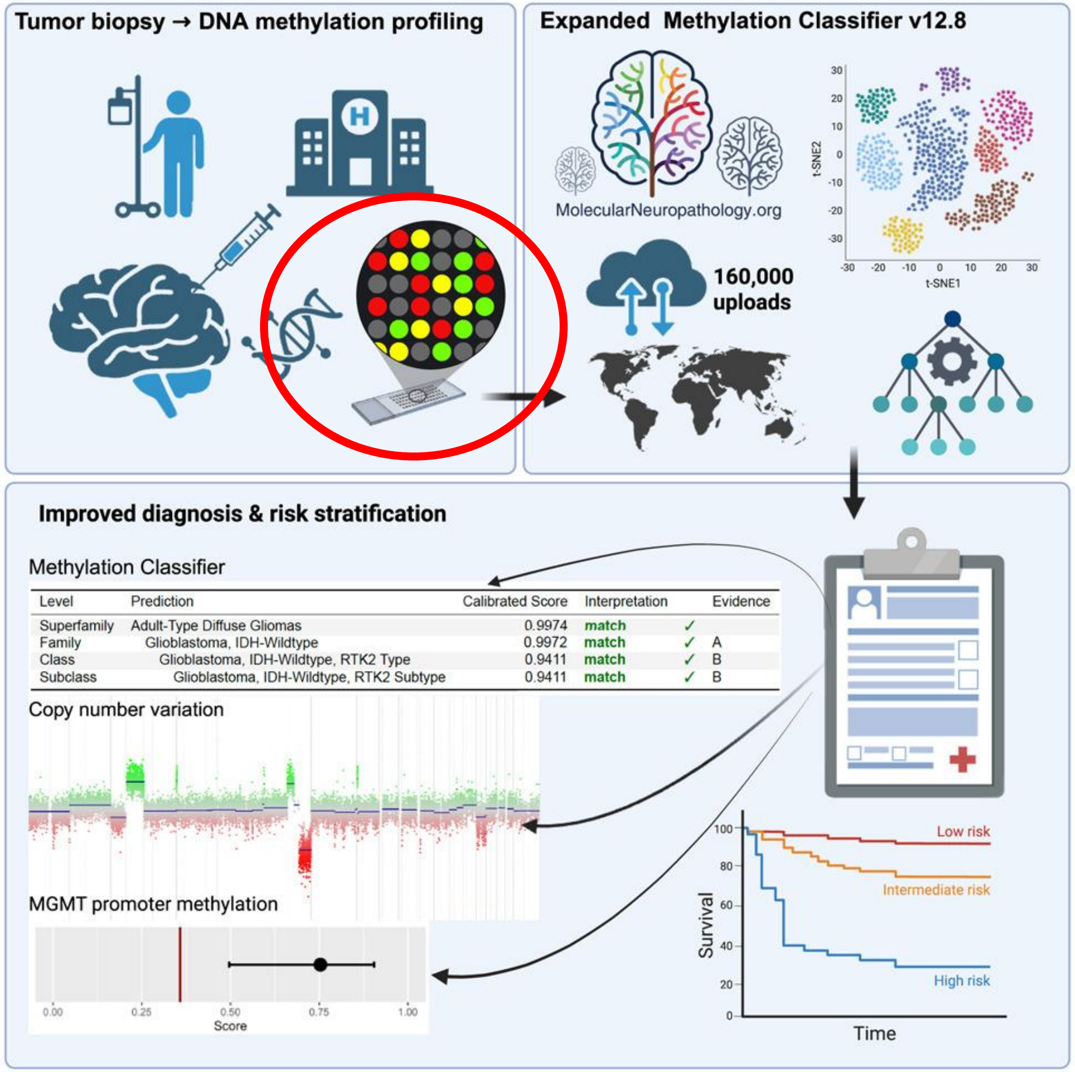

| Level | Prediction | Calibrated Score | Interpretation | | Evidence |
|---|---|---|---|---|---|
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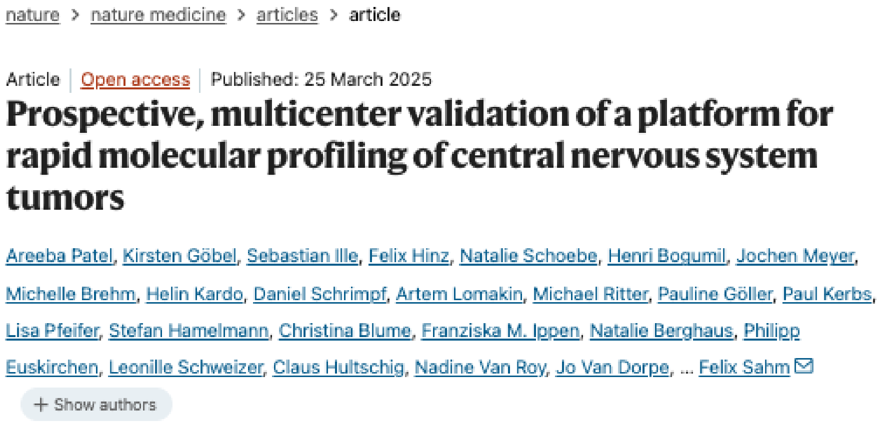
| Class | Glioblastoma, IDH-Wildtype, RTK2 Type | 0.9411 | match | ✓ | B |
| Subclass | Glioblastoma, IDH-Wildtype, RTK2 Subtype | 0.9411 | match | ✓ | B |

*Sill et al. 2025*

# MNP-flex: a platform agnostic classifier for CNS tumors



nature > nature medicine > articles > article

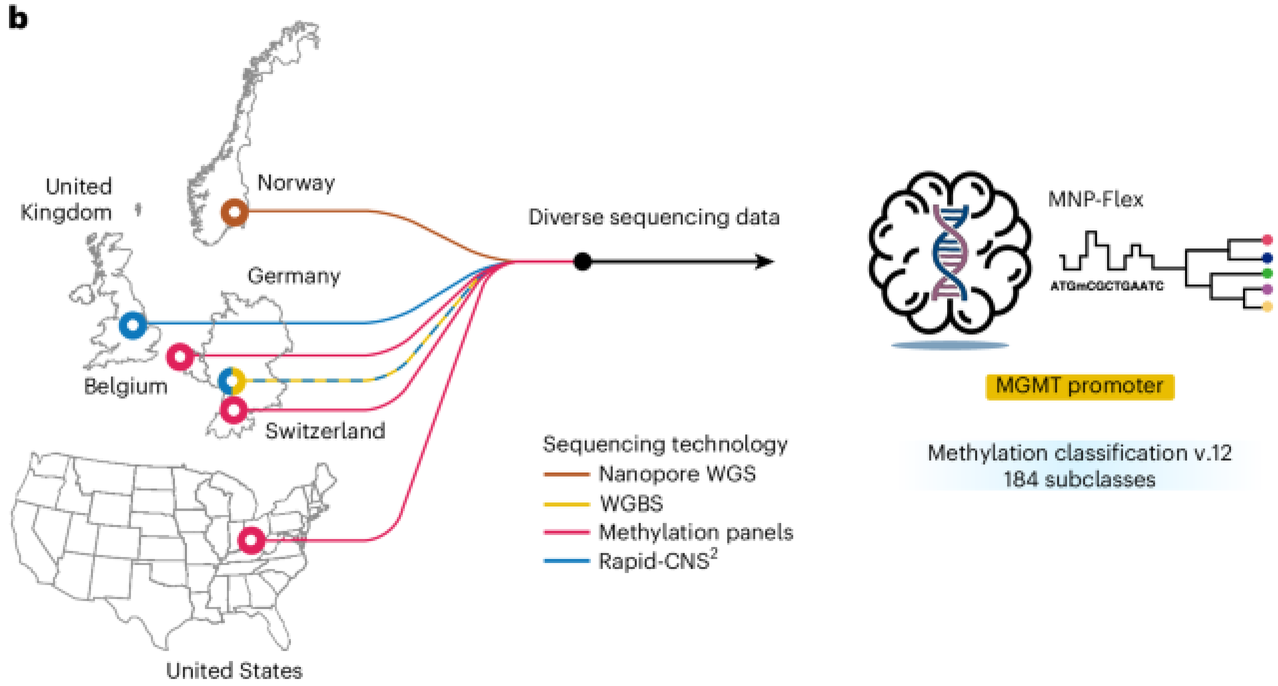
Article | Open access | Published: 25 March 2025

**Prospective, multicenter validation of a platform for rapid molecular profiling of central nervous system tumors**

Areeba Patel, Kirsten Göbel, Sebastian Ille, Felix Hinz, Natalie Schoebe, Henri Bogumil, Jochen Meyer, Michelle Brehm, Helin Kardo, Daniel Schrimpf, Artem Lomakin, Michael Ritter, Pauline Göller, Paul Kerbs, Lisa Pfeifer, Stefan Hamelmann, Christina Blume, Franziska M. Ippen, Natalie Berghaus, Philipp Euskirchen, Leonille Schweizer, Claus Hultschig, Nadine Van Roy, Jo Van Dorpe, ... Felix Sahm

+ Show authors

- Uses the entire MNP dataset to train new model
  - Only samples with score >0.7 included
  - Binarized methylation values to compensate for nuances of the different technologies
- Test the model on 448 samples of 5 different technologies



Number of samples:
- Nanopore WGS: 40
- WGBS: 80
- Methylation panels: 27
- Rapid-CNS:301

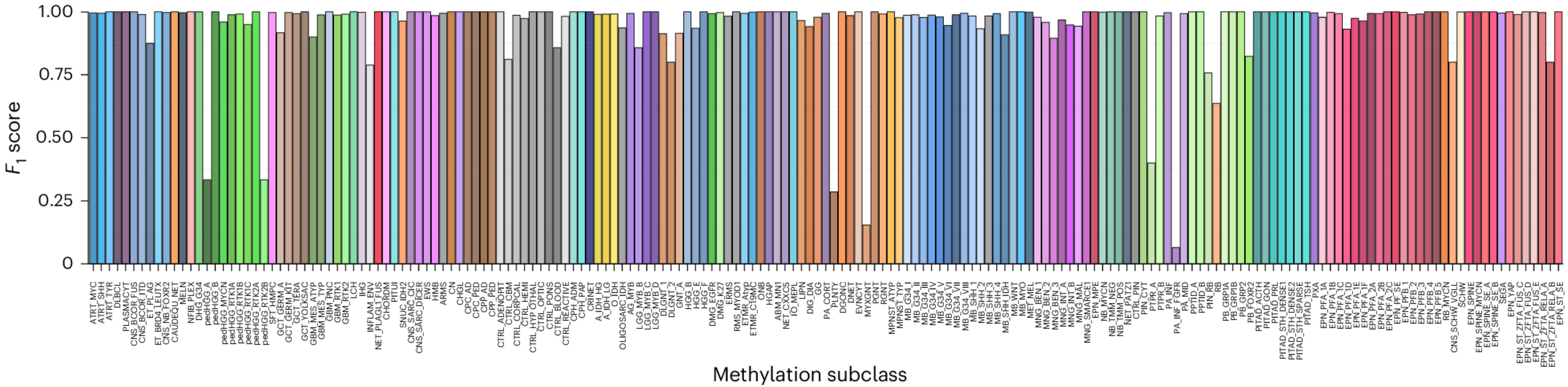
*Patel et al.,2025*

# MNP-flex: a reliable platform agnostic model

176/182 subclasses show a F1 score >0.5

163/182 subclasses show a F1 score >0.9

Low scores are found for subclasses with low reference samples

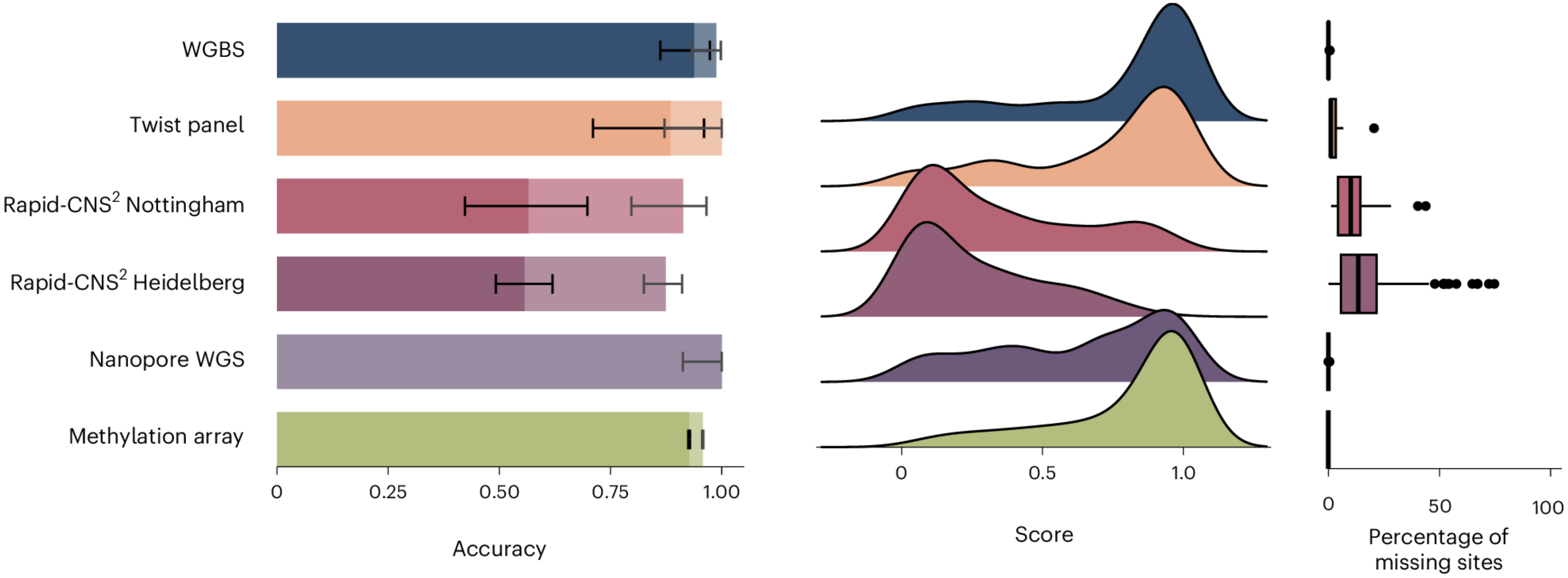


*Patel et al.,2025*

# MNP-flex: a reliable platform agnostic model

With a cut off of **0.3** for MNP-flex score:

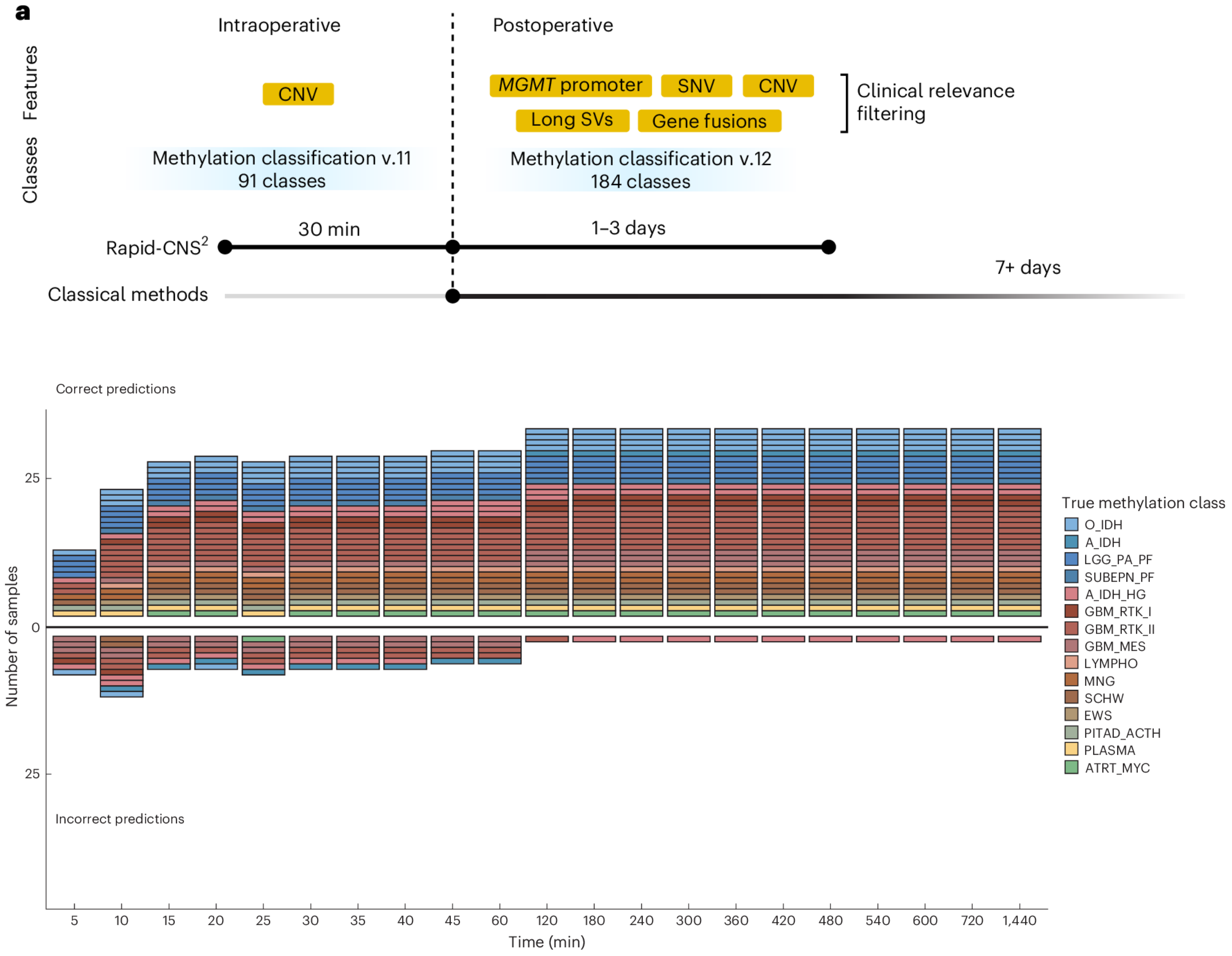
- 99.6% accuracy for methylation families
- 99.2 for methylation subclass



Lower scores are associated with more missing sites

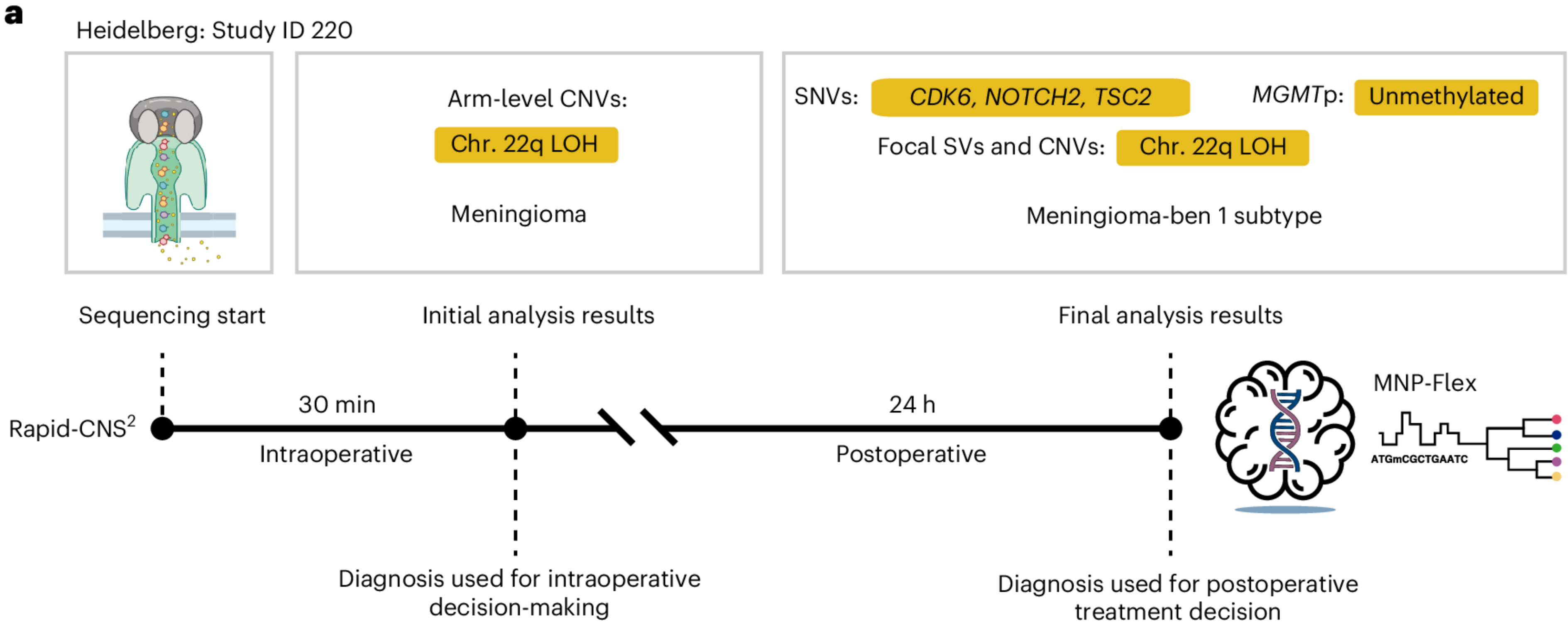
*Patel et al.,2025*

# Integration of Rapid-CNS and MNP flex



*Patel et al.,2025*

# Integration of Rapid-CNS and MNP flex



**a**

Heidelberg: Study ID 220

Arm-level CNVs: Chr. 22q LOH

Meningioma

SNVs: *CDK6, NOTCH2, TSC2*

*MGMT*p: Unmethylated

Focal SVs and CNVs: Chr. 22q LOH

Meningioma-ben 1 subtype

Sequencing start

Initial analysis results

Final analysis results

Rapid-CNS$^2$

30 min

Intraoperative

24 h

Postoperative

MNP-Flex

Diagnosis used for intraoperative decision-making

Diagnosis used for postoperative treatment decision

*Patel et al.,2025*

| | MNP (v12.8)/Epignostix | Rapid CNS$^2$ | Sturgeon | MNP-flex |
|---|---|---|---|---|
| | Most widely used, based on EPIC array | Faster and low throughput version of the MNP v11 based on nanopore | Build for **sparse** data | The golden standard MNP classifier for all platforms |
| Use case | Golden standard | Fast results, during surgery possible | During surgery | Post surgery, non array data |
| Training data | 7495 methylation profiles | MNP v11 | MNP v11 | MNP v12.8 |
| n (sub)classes | 184 | 91 | 91 | 184 |
| Technique | Illumina EPIC array | Nanopore | Nanopore | Platform agnostic |
| | | Adaptive sampling | Adaptive sampling optional | (WGBS, nanpore, targeted methylation seq) |
| Turn around time | 2 weeks | 4-5 days | 90 min | Depeding on technique – days -> weeks |
| Accessibility | Webbased<br>https://app.epignostix.com | Github, Nextflow | Github | Webbased<br>https://mnp-flex.org/ |
| Input files | IDAT | modBams | modBams | Bed file, EPIC CpG sites, coverage, methylation percentage |
| Output | Methylation class<br>CNV profile<br>MGMT | Methylation class<br>CNV profile<br>MGMT<br>SNV, SV | Methylation class<br>Live tool available (classification during sequencing) | Methylation class<br>MGMT |
| Publication | Capper et al., 2018, Sill et al., 2025 | Patel et al., 2022,2025 | Vermeulen et al., 2023 | Patel et al., 2025 |

J Korean Med Sci. 2023 Oct 23;38(43):e356. doi: 10.3346/jkms.2023.38.e356

## Brain Tumor Classification by Methylation Profile

Jin Woo Park[1,2,*], Kwanghoon Lee[1,*], Eric Eunshik Kim[1], Seong-Ik Kim[1], Sung-Hye Park[1,3,✉]

Author information Article notes Copyright and License information

PMCID: PMC10627723 PMID: 37935168

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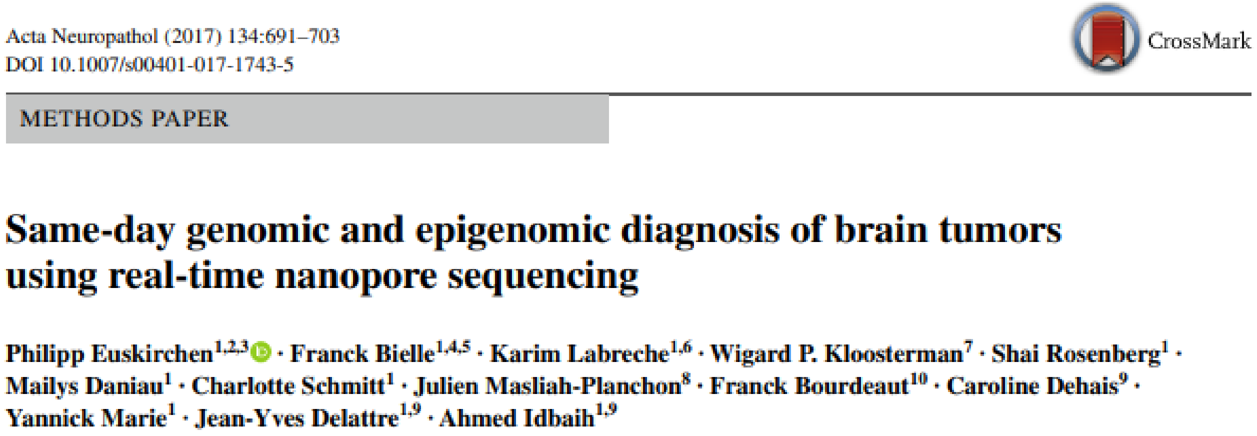

Acta Neuropathol (2017) 134:691–703
DOI 10.1007/s00401-017-1743-5

CrossMark

METHODS PAPER

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

Philipp Euskirchen[1,2,3] · Franck Bielle[1,4,5] · Karim Labreche[1,6] · Wigard P. Kloosterman[7] · Shai Rosenberg[1] · Mailys Daniau[1] · Charlotte Schmitt[1] · Julien Masliah-Planchon[8] · Franck Bourdeaut[10] · Caroline Dehais[9] · Yannick Marie[1] · Jean-Yves Delattre[1,9] · Ahmed Idbaih[1,9]

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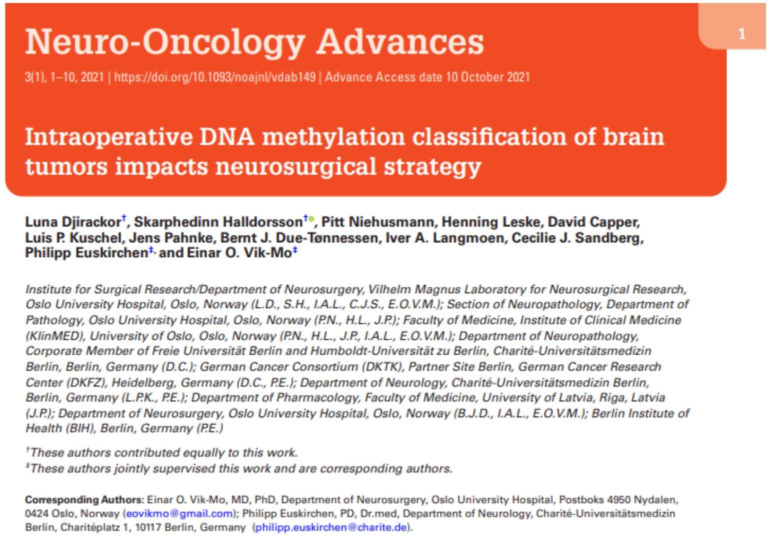

Neuro-Oncology Advances

3(1), 1–10, 2021 | https://doi.org/10.1093/noajnl/vdab149 | Advance Access date 10 October 2021

1

## Intraoperative DNA methylation classification of brain tumors impacts neurosurgical strategy

Luna Djirackor[†], Skarphedinn Halldorsson[†], Pitt Niehusmann, Henning Leske, David Capper, Luis P. Kuschel, Jens Pahnke, Bernt J. Due-Tønnessen, Iver A. Langmoen, Cecilie J. Sandberg, Philipp Euskirchen[‡], and Einar O. Vik-Mo[‡]

*Institute for Surgical Research/Department of Neurosurgery, Vilhelm Magnus Laboratory for Neurosurgical Research, Oslo University Hospital, Oslo, Norway (L.D., S.H., I.A.L., C.J.S., E.O.V.M.); Section of Neuropathology, Department of Pathology, Oslo University Hospital, Oslo, Norway (P.N., H.L., J.P.); Faculty of Medicine, Institute of Clinical Medicine (KlinMED), University of Oslo, Oslo, Norway (P.N., H.L., J.P., I.A.L., E.O.V.M.); Department of Neuropathology, Corporate Member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Charité-Universitätsmedizin Berlin, Berlin, Germany (D.C.); German Cancer Consortium (DKTK), Partner Site Berlin, German Cancer Research Center (DKFZ), Heidelberg, Germany (D.C., P.E.); Department of Neurology, Charité-Universitätsmedizin Berlin, Berlin, Germany (L.P.K., P.E.); Department of Pharmacology, Faculty of Medicine, University of Latvia, Riga, Latvia (J.P.); Department of Neurosurgery, Oslo University Hospital, Oslo, Norway (B.J.D., I.A.L., E.O.V.M.); Berlin Institute of Health (BIH), Berlin, Germany (P.E.)*

[†] *These authors contributed equally to this work.*

[‡] *These authors jointly supervised this work and are corresponding authors.*

**Corresponding Authors:** Einar O. Vik-Mo, MD, PhD, Department of Neurosurgery, Oslo University Hospital, Postboks 4950 Nydalen, 0424 Oslo, Norway (eovikmo@gmail.com); Philipp Euskirchen, PD, Dr.med, Department of Neurology, Charité-Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany (philipp.euskirchen@charite.de).

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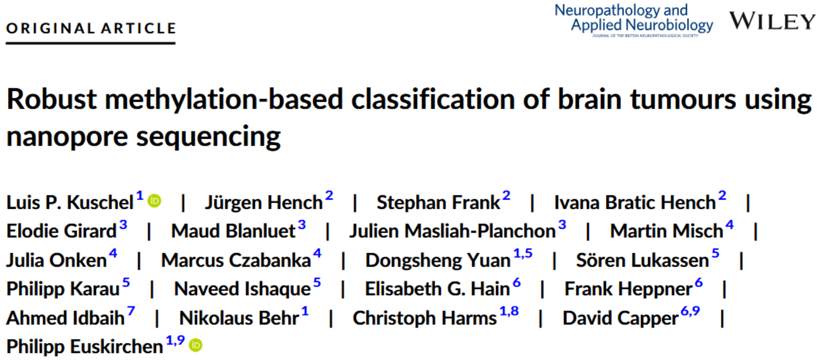

ORIGINAL ARTICLE

Neuropathology and Applied Neurobiology WILEY

## Robust methylation-based classification of brain tumours using nanopore sequencing

Luis P. Kuschel[1] | Jürgen Hench[2] | Stephan Frank[2] | Ivana Bratic Hench[2] | Elodie Girard[3] | Maud Blanluet[3] | Julien Masliah-Planchon[3] | Martin Misch[4] | Julia Onken[4] | Marcus Czabanka[4] | Dongsheng Yuan[1,5] | Sören Lukassen[5] | Philipp Karau[5] | Naveed Ishaque[5] | Elisabeth G. Hain[6] | Frank Heppner[6] | Ahmed Idbaih[7] | Nikolaus Behr[1] | Christoph Harms[1,8] | David Capper[6,9] | Philipp Euskirchen[1,9]

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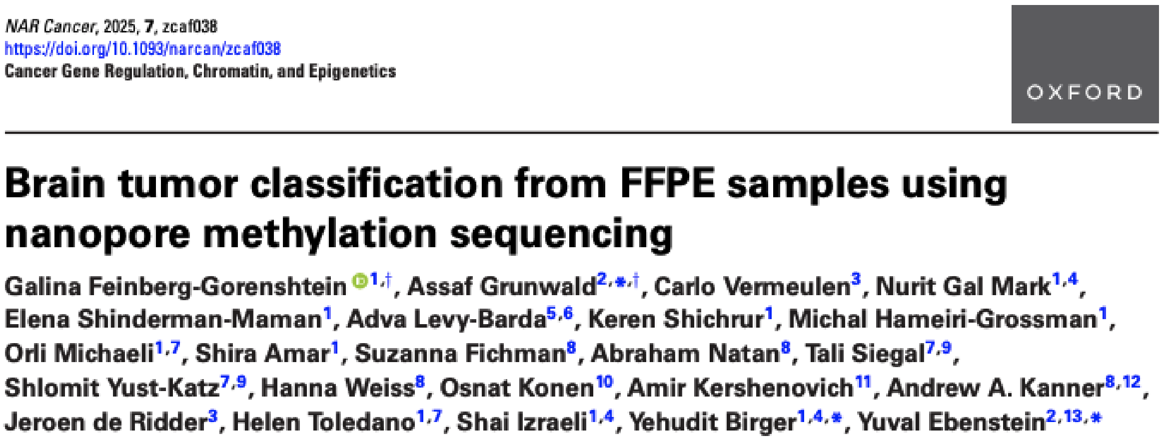


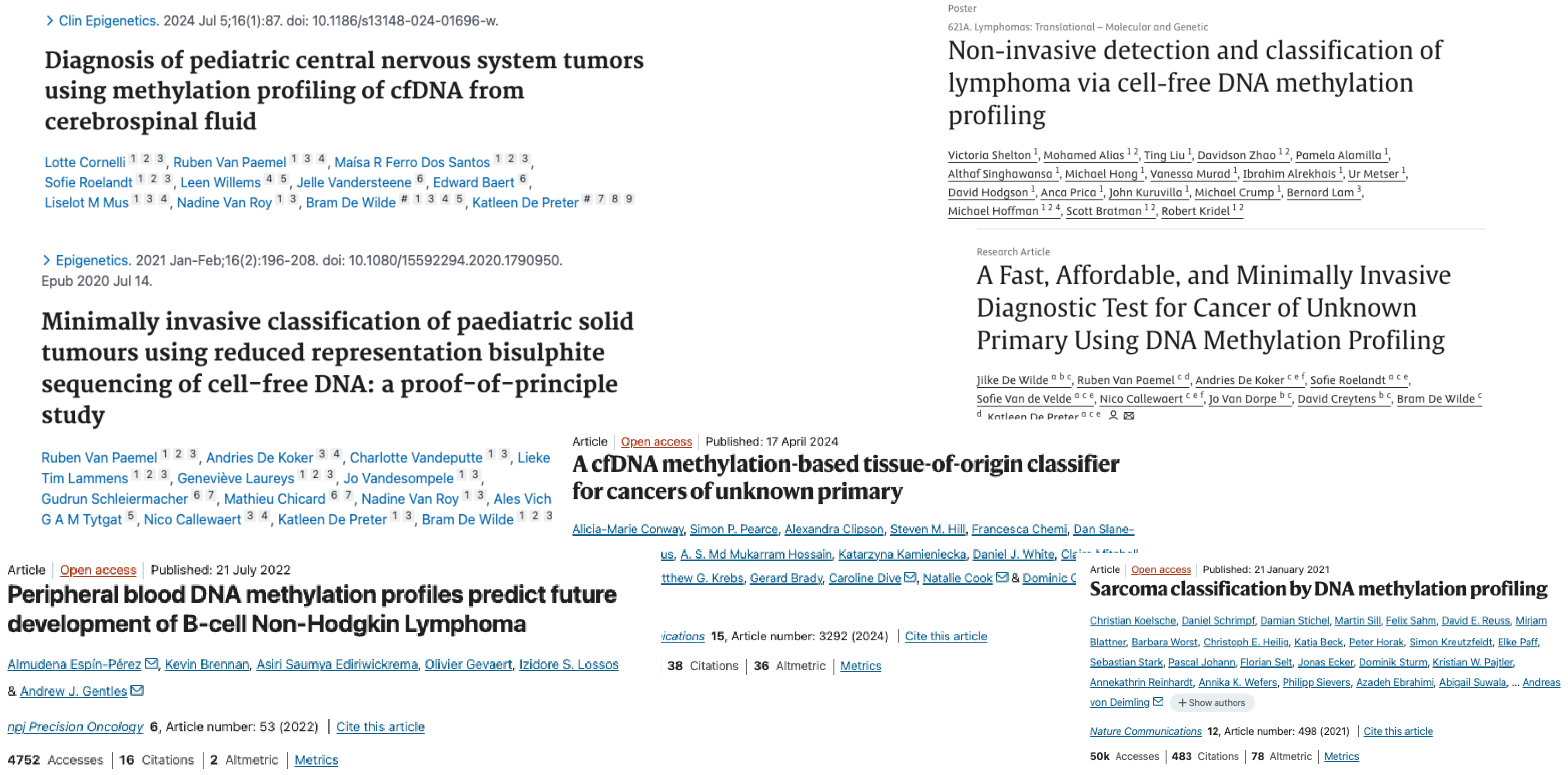
*NAR Cancer*, 2025, **7**, zcaf038
https://doi.org/10.1093/narcan/zcaf038
**Cancer Gene Regulation, Chromatin, and Epigenetics**

OXFORD

## Brain tumor classification from FFPE samples using nanopore methylation sequencing

Galina Feinberg-Gorenshtein[1,†], Assaf Grunwald[2,*,†], Carlo Vermeulen[3], Nurit Gal Mark[1,4], Elena Shinderman-Maman[1], Adva Levy-Barda[5,6], Keren Shichrur[1], Michal Hameiri-Grossman[1], Orli Michaeli[1,7], Shira Amar[1], Suzanna Fichman[8], Abraham Natan[8], Tali Siegal[7,9], Shlomit Yust-Katz[7,9], Hanna Weiss[8], Osnat Konen[10], Amir Kershenovich[11], Andrew A. Kanner[8,12], Jeroen de Ridder[3], Helen Toledano[1,7], Shai Izraeli[1,4], Yehudit Birger[1,4,*], Yuval Ebenstein[2,13,*]

# Methylation: diagnostically important for many tumor types



> Clin Epigenetics. 2024 Jul 5;16(1):87. doi: 10.1186/s13148-024-01696-w.

**Diagnosis of pediatric central nervous system tumors using methylation profiling of cfDNA from cerebrospinal fluid**

Lotte Cornelli[1,2,3], Ruben Van Paemel[1,3,4], Maísa R Ferro Dos Santos[1,2,3], Sofie Roelandt[1,2,3], Leen Willems[4,5], Jelle Vandersteene[6], Edward Baert[6], Liselot M Mus[1,3,4], Nadine Van Roy[1,3], Bram De Wilde[#,1,3,4,5], Katleen De Preter[#,7,8,9]

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Poster

621A. Lymphomas: Translational – Molecular and Genetic

**Non-invasive detection and classification of lymphoma via cell-free DNA methylation profiling**

Victoria Shelton[1], Mohamed Alias[1,2], Ting Liu[1], Davidson Zhao[1,2], Pamela Alamilla[1], Althaf Singhawansa[1], Michael Hong[1], Vanessa Murad[1], Ibrahim Alrekhais[1], Ur Metser[1], David Hodgson[1], Anca Prica[1], John Kuruvilla[1], Michael Crump[1], Bernard Lam[3], Michael Hoffman[1,2,4], Scott Bratman[1,2], Robert Kridel[1,2]

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> Epigenetics. 2021 Jan-Feb;16(2):196-208. doi: 10.1080/15592294.2020.1790950.
Epub 2020 Jul 14.

**Minimally invasive classification of paediatric solid tumours using reduced representation bisulphite sequencing of cell-free DNA: a proof-of-principle study**

Ruben Van Paemel[1,2,3], Andries De Koker[3,4], Charlotte Vandeputte[1,3], Lieke Tim Lammens[1,2,3], Geneviève Laureys[1,2,3], Jo Vandesompele[1,3], Gudrun Schleiermacher[6,7], Mathieu Chicard[6,7], Nadine Van Roy[1,3], Ales Vich G A M Tytgat[5], Nico Callewaert[3,4], Katleen De Preter[1,3], Bram De Wilde[1,2,3]

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Research Article

**A Fast, Affordable, and Minimally Invasive Diagnostic Test for Cancer of Unknown Primary Using DNA Methylation Profiling**

Jilke De Wilde[a,b,c], Ruben Van Paemel[c,d], Andries De Koker[c,e,f], Sofie Roelandt[a,c,e], Sofie Van de Velde[a,c,e], Nico Callewaert[c,e,f], Jo Van Dorpe[b,c], David Creytens[b,c], Bram De Wilde[c,d], Katleen De Preter[a,c,e]

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Article | Open access | Published: 17 April 2024

**A cfDNA methylation-based tissue-of-origin classifier for cancers of unknown primary**

Alicia-Marie Conway, Simon P. Pearce, Alexandra Clipson, Steven M. Hill, Francesca Chemi, Dan Slane-
us, A. S. Md Mukarram Hossain, Katarzyna Kamieniecka, Daniel J. White, Cla
tthew G. Krebs, Gerard Brady, Caroline Dive, Natalie Cook & Dominic

ications 15, Article number: 3292 (2024) | Cite this article

38 Citations | 36 Altmetric | Metrics

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Article | Open access | Published: 21 July 2022

**Peripheral blood DNA methylation profiles predict future development of B-cell Non-Hodgkin Lymphoma**

Almudena Espín-Pérez, Kevin Brennan, Asiri Saumya Ediriwickrema, Olivier Gevaert, Izidore S. Lossos & Andrew J. Gentles

*npj Precision Oncology* 6, Article number: 53 (2022) | Cite this article

4752 Accesses | 16 Citations | 2 Altmetric | Metrics

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Article | Open access | Published: 21 January 2021

**Sarcoma classification by DNA methylation profiling**

Christian Koelsche, Daniel Schrimpf, Damian Stichel, Martin Sill, Felix Sahm, David E. Reuss, Mirjam Blattner, Barbara Worst, Christoph E. Heilig, Katja Beck, Peter Horak, Simon Kreutzfeldt, Elke Paff, Sebastian Stark, Pascal Johann, Florian Selt, Jonas Ecker, Dominik Sturm, Kristian W. Pajtler, Annekathrin Reinhardt, Annika K. Wefers, Philipp Sievers, Azadeh Ebrahimi, Abigail Suwala, ... Andreas von Deimling

*Nature Communications* 12, Article number: 498 (2021) | Cite this article

50k Accesses | 483 Citations | 78 Altmetric | Metrics

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