Rigshospitalet
The Centre of Diagnostic Investigations

Nanopore sequensing for rapid and precise classification of CNS tumors

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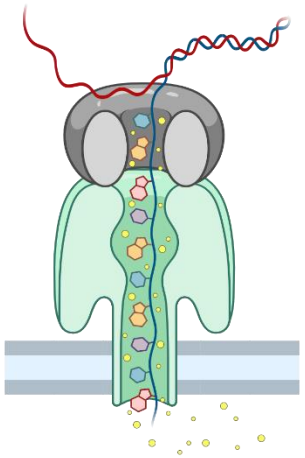
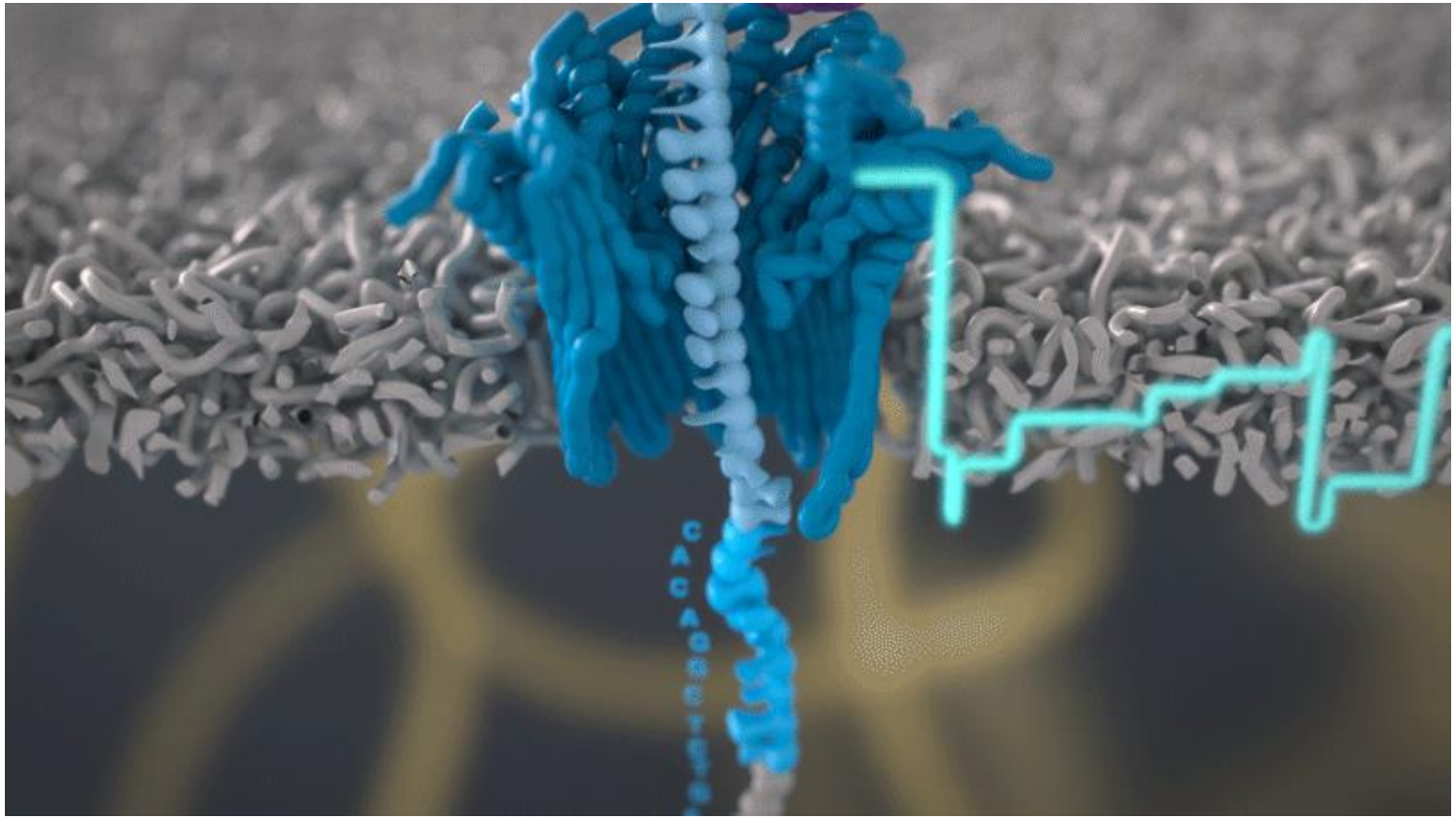
Nanopore sequencing

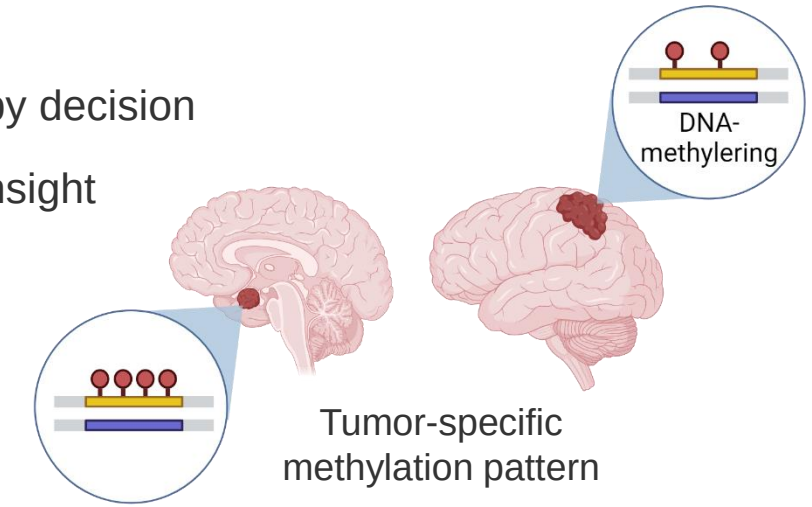


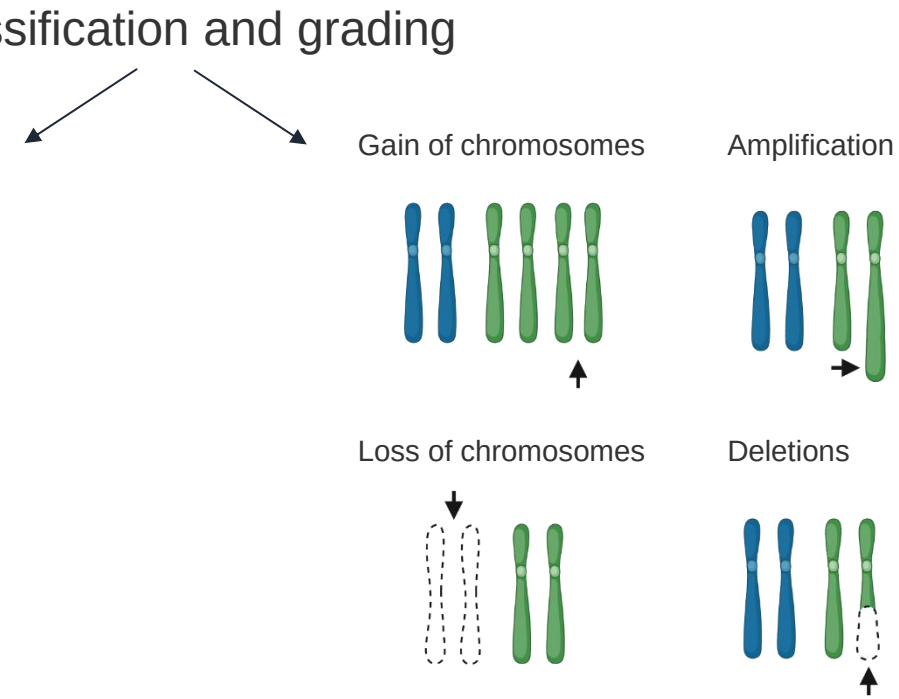
Sequencing
with Nanopore



Methylation-based classification of CNS tumors

- >100 known CNS tumor types
- Why classify?
  - Guide therapy decision
  - Prognostic insight





- Since 2018: EPIC - array-based methylation analysis
- Heidelberg CNS tumor classifier
- Time-consuming (6-10 working days + fixation and embedding)

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

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]

Neuro-Oncology Advances

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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[‡]

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Neuropathology and Applied Neurobiology
JOURNAL OF THE BRITISH NEUROPATHOLOGICAL SOCIETY

WILEY

ORIGINAL ARTICLE

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]

Article

Ultra-fast deep-learned CNS tumour classification during surgery

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C. Vermeulen[1,2,6], M. Pagès-Gallego[1,2,6], L. Kester[3], M. E. G. Kranendonk[3], P. Wesseling[3,4], N. Verburg[5], P. de Witt Hamer[5], E. J. Kooi[4], L. Dankmeijer[4,5], J. van der Lugt[3], K. van Baarsen[3], E. W. Hoving[3], B. B. J. Tops[3]✉ & J. de Ridder[1,2]✉

JOURNAL ARTICLE

cIMPACT-NOW update 8: Clarifications on molecular risk parameters and recommendations for WHO grading of meningiomas

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Felix Sahm ✉, Kenneth D Aldape, Priscilla K Brastianos, Daniel J Brat, Sonika Dahiya, Andreas von Deimling, Caterina Giannini, Mark R Gilbert, David N Louis, David R Raleigh
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Neuro-Oncology, Volume 27, Issue 2, February 2025, Pages 319–330,
https://doi.org/10.1093/neuonc/noae170
Published: 30 August 2024 **Article history** ▾

Combined 1p/22q loss;
WHO grade 2

Visit to Berlin, December 2023

Dr. Philipp Euskirchen
Institut für Neuropathologie
Charité - Universitätsmedizin Berlin

Nanopore sequencing

- Short to ultra-long reads
- Native DNA or RNA
- No PCR required
- Methylation information (no bisulfite conversion)
- Real time data availability
- Targeted sequencing possible

Flow cell

Images from nanoporetech.com

NanoDx analysis runs daily

Urgent samples < 5 h

3-4 cases daily (one flow-cell)

Workflow

Surgery

Delivery to ”FRYS” team

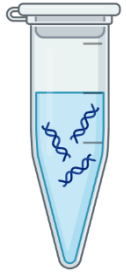
Microtomy

Sample for molecular analysis

Neuropathologist examining HE and VG stainings

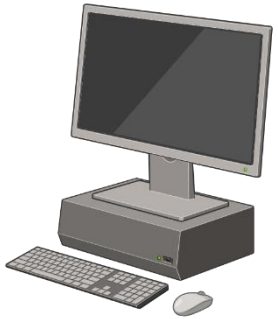
Transfer to molecular lab

Homogenization



DNA extraction and library prep

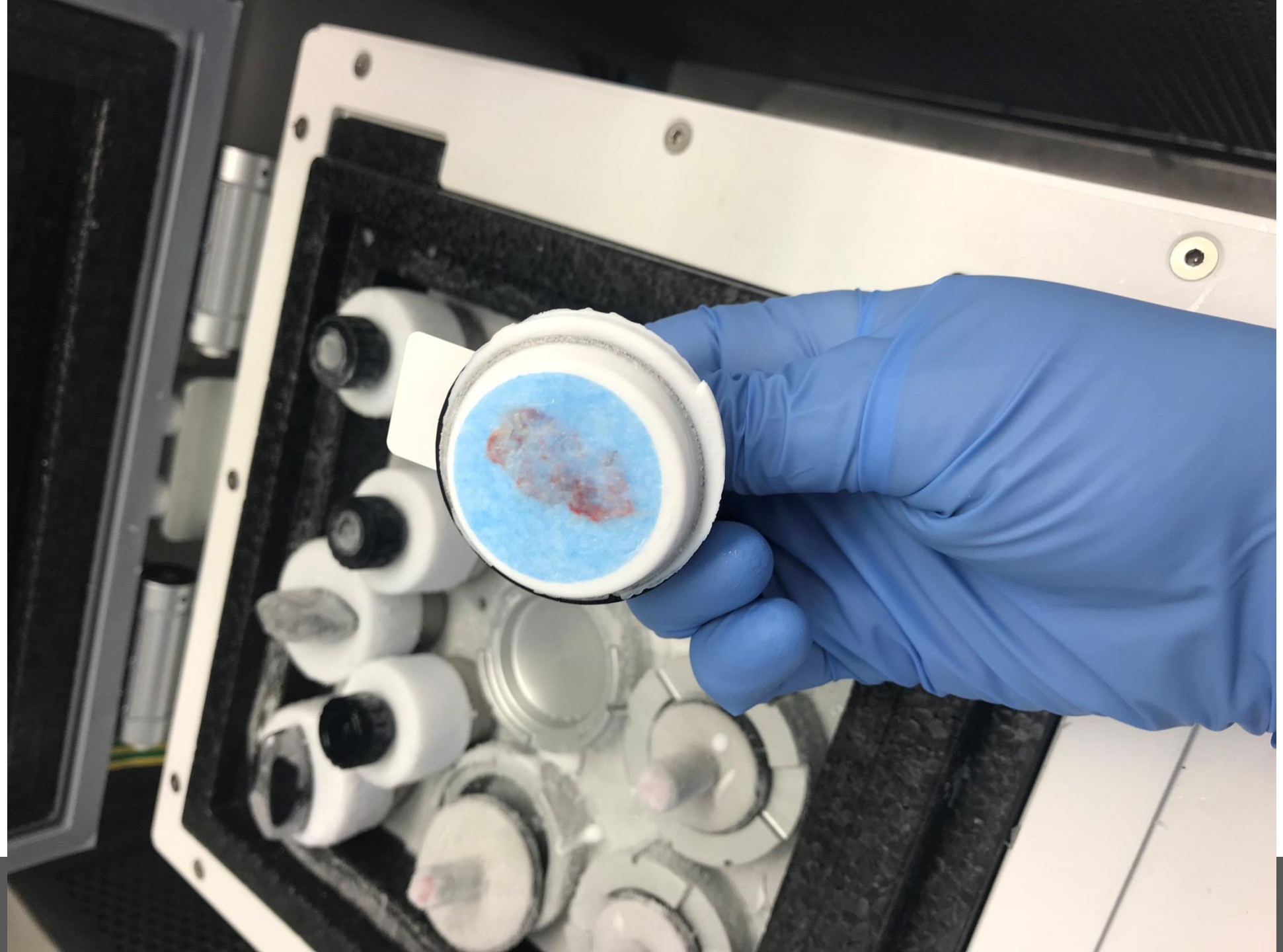
Sequencing on GridION

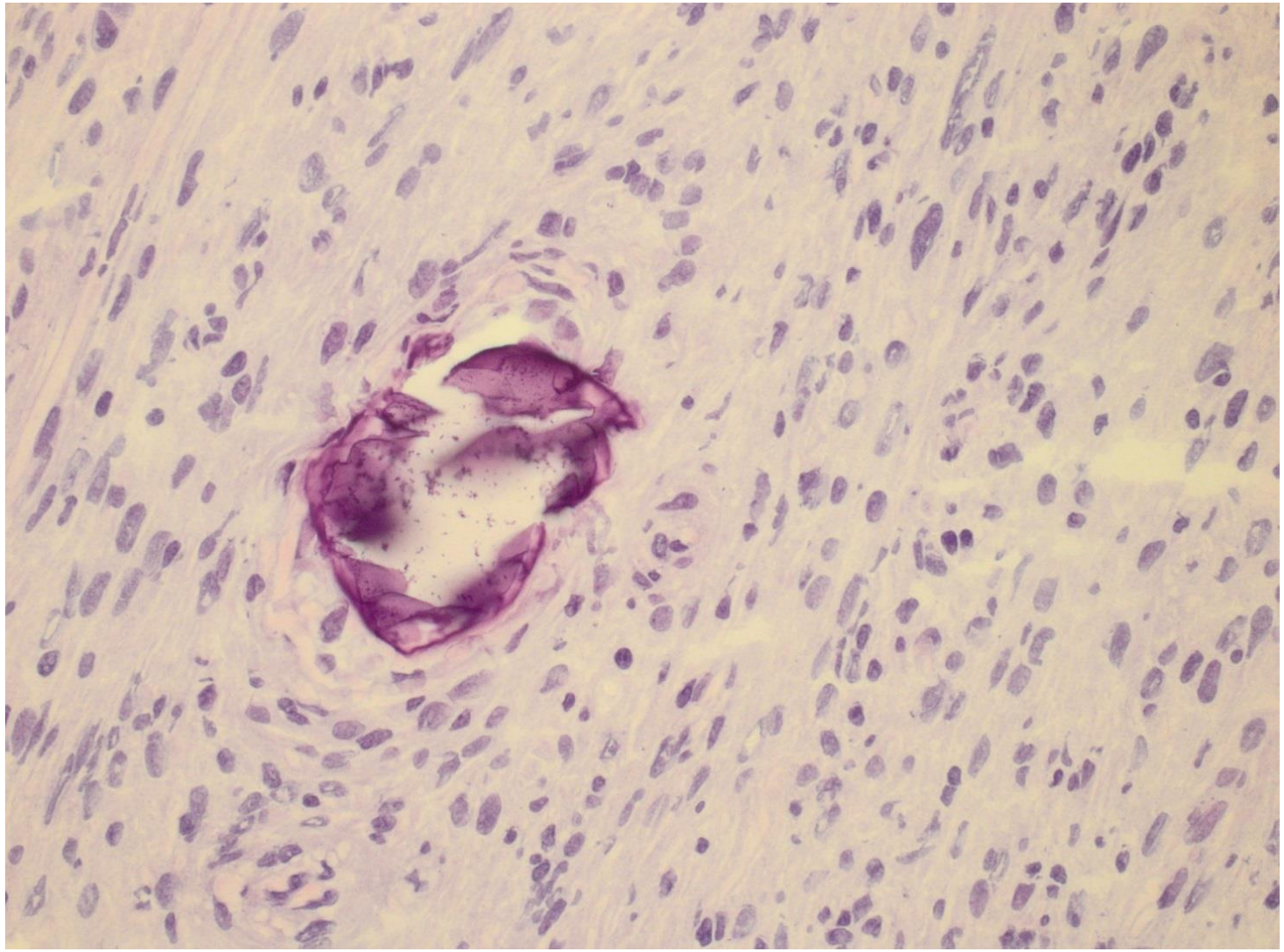


nanoDx data analysis

Interpretation of results

3 h | **2-16 h** | **20 min**

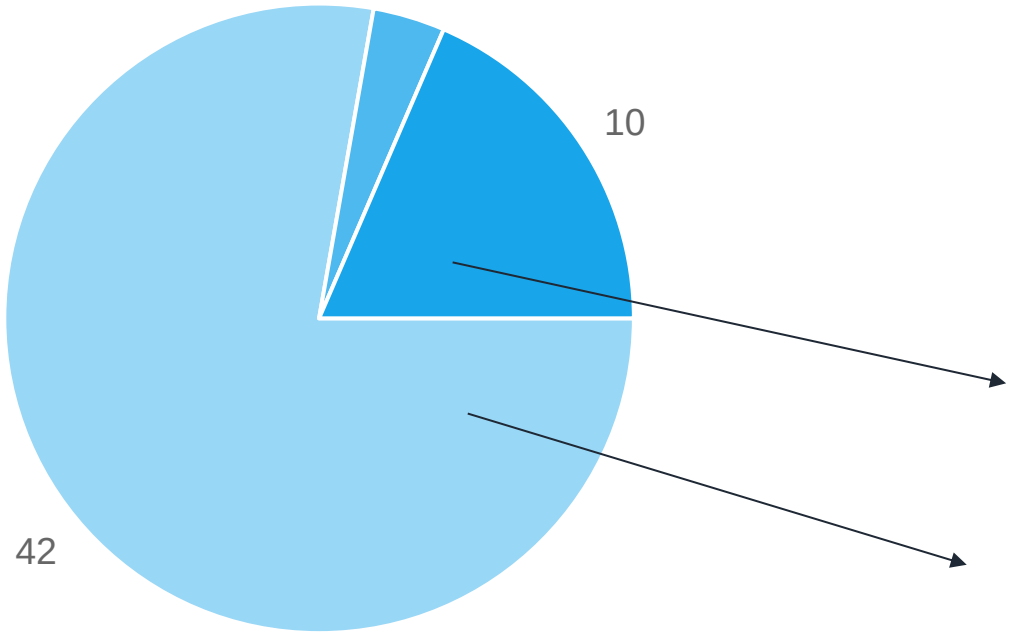




Validation period, June 2024

Inclusion: Every CNS sample sent for frozen; gliomas, meningiomas, metastases, lymphomas, pituitary adenomas etc

nanoDx vs. final diagnosis, 54 samples



Two cases with score >0.2 for ”control tissue, reactive tumor environment”

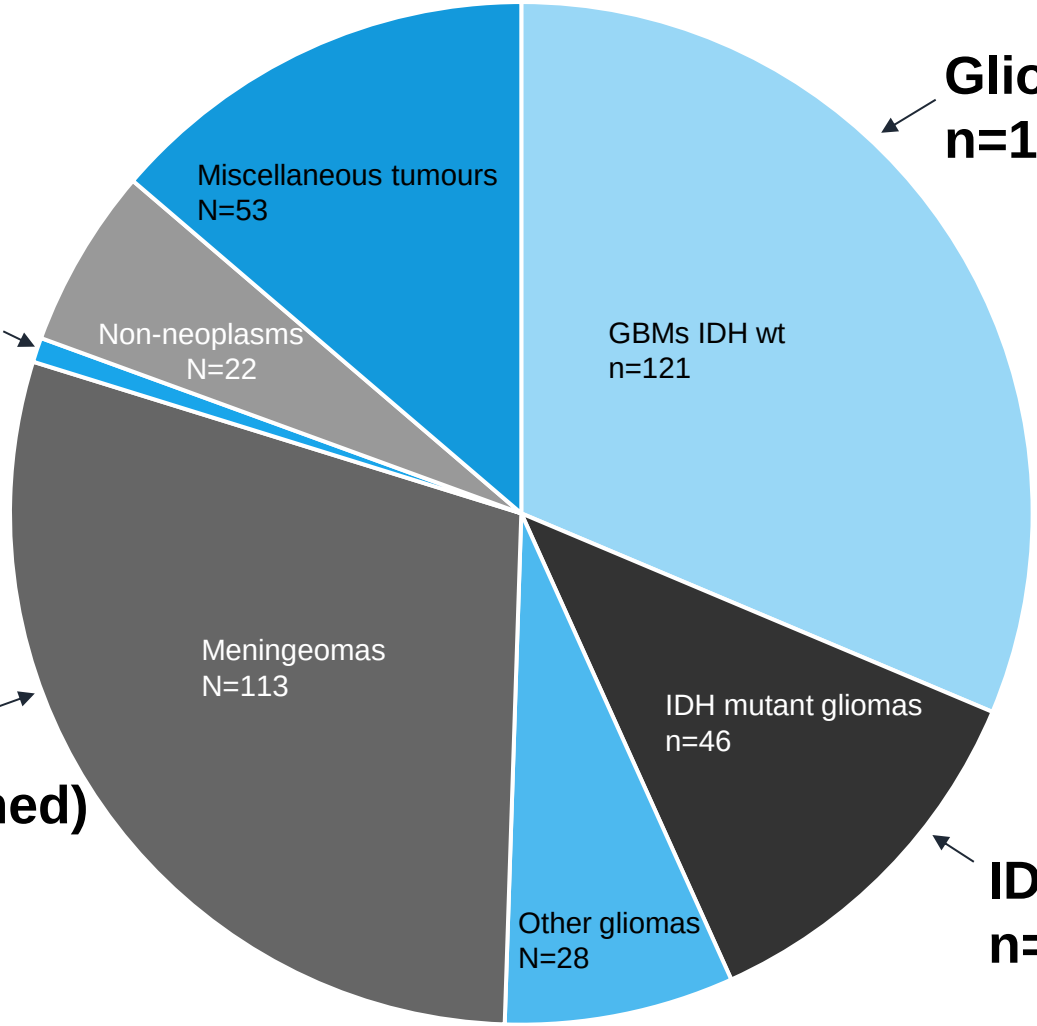
- Craniopharyngeoma
- Carcinoma metastasis

No match, score <0.2

Match, score >0.2 and concurrent with the final diagnosis

Diagnostic routine from August 2024 to April 2025, 386 cases: Nanopore results versus final diagnoses

Indication: menigioma, glioma, pediatric



Glioblastomas, IDH-wildtype, n=110/121 (90% matched)

Embryonal tumors, n=3/3 (100%) matched

Meningeomas, n=111/113 (98% matched)

IDH-mutant gliomas, n=45/46 (98% matched)

Nanopore results vs. final integrated diagnoses

- 5/386 cases were technically unsuccessful
- 65 /386 (17 %) were non-classifiable, largest group being non-neoplastic cases followed by glioblastomas (GBMs) IDH wt and metastases
- GBMs, IDH wt, n=110/121 (90% matched)
- When GBMs did not match to an entity, it was typically due to low tumor content/reactive microenvironment.
- IDH-mutant gliomas, n=45/46 (98% matched) . In all gliomas 45/45 (100%) classified as IDH-mutant, the IDH mutation was confirmed
- 3 cases n=3/386 (0,5%) did match to a wrong entity; a pituitary adenoma classified as an esthesioneuroblastoma, a reactive case classified as a meningioma, a fossa posterior ependymoma WHO grade 3 (TERT mutation monosomy 6) classified as subependymoma.

Nanopore results vs. final integrated diagnoses, other highlights

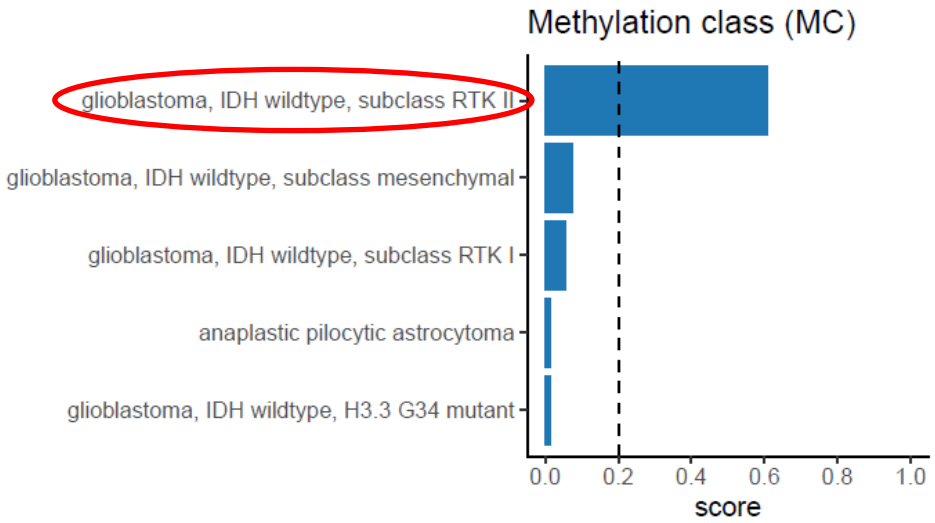
- 33/113 (29%) of meningiomas had 1p/22q loss, ie WHO grade 2
- 2/2 Diffuse midline glioma, H3 K27 altered were correctly classified
- 3/3 embryonal tumors correctly classified; ETMR, medulloblastoma-wnt, medulloblastoma group 3. All confirmed by Epic.
- 3/5 ependymomas correctly classified. One misleading; subepenymoma fossa posterior vs ependymoma WHO grade 3 (TERT mutation and monosomy 6)

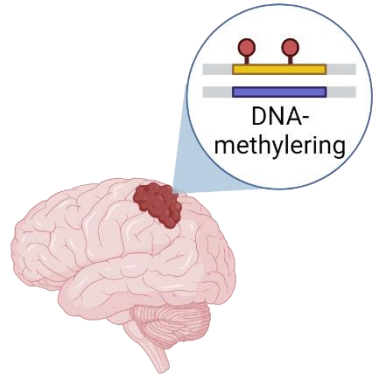
nanoDx pipeline

Uses Heidelberg's (German Cancer Research Center) CNS tumor classifier version 11b4

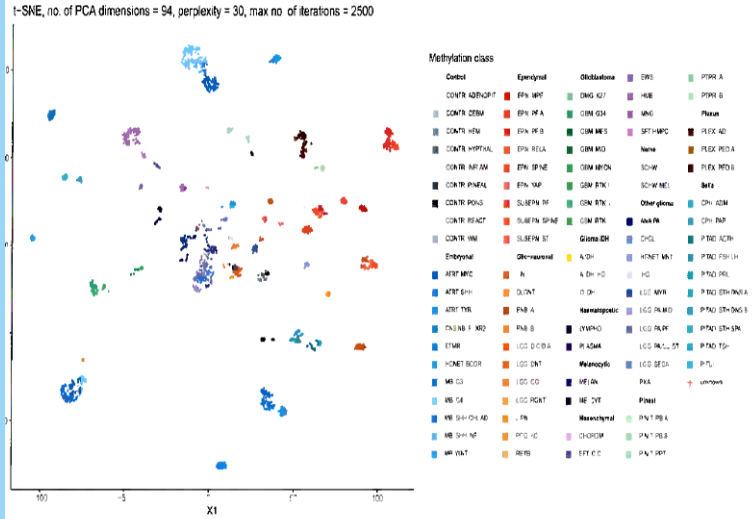
Euskirchen et al. (Charité, Berlin)

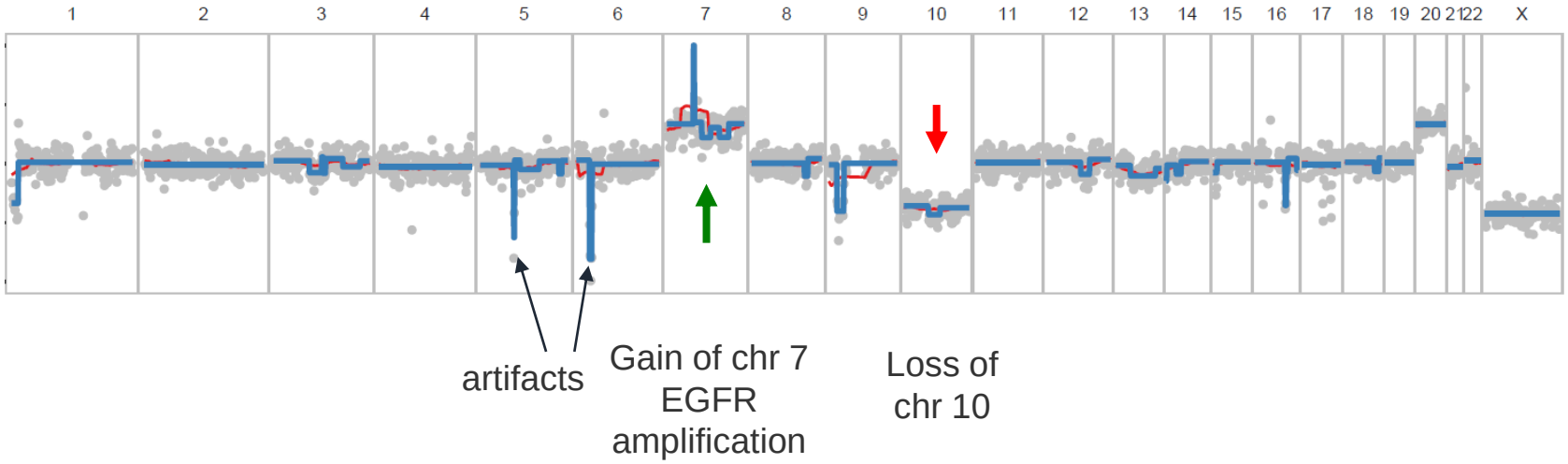
Glioblastoma IDH wt



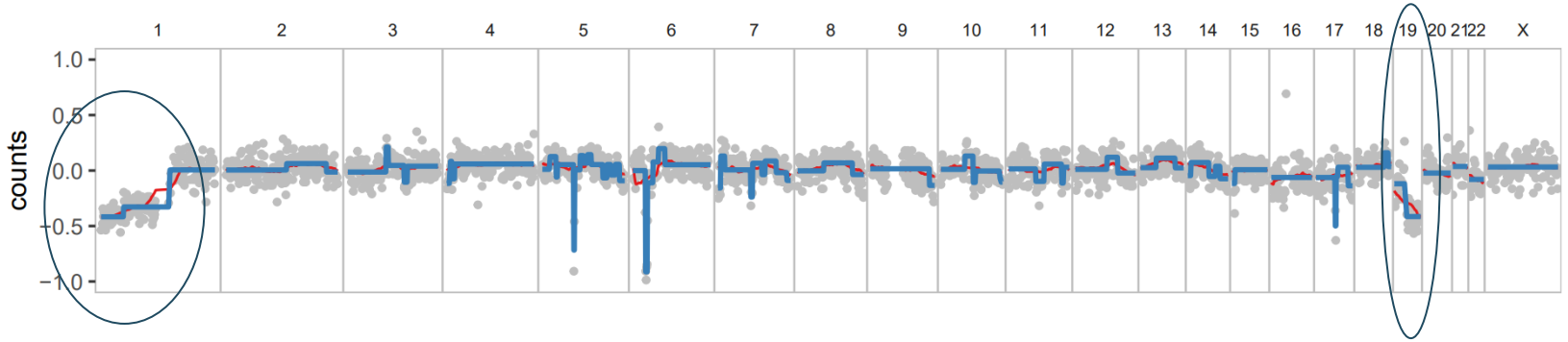


Methylation analysis
Match >0.2





Copy number profile

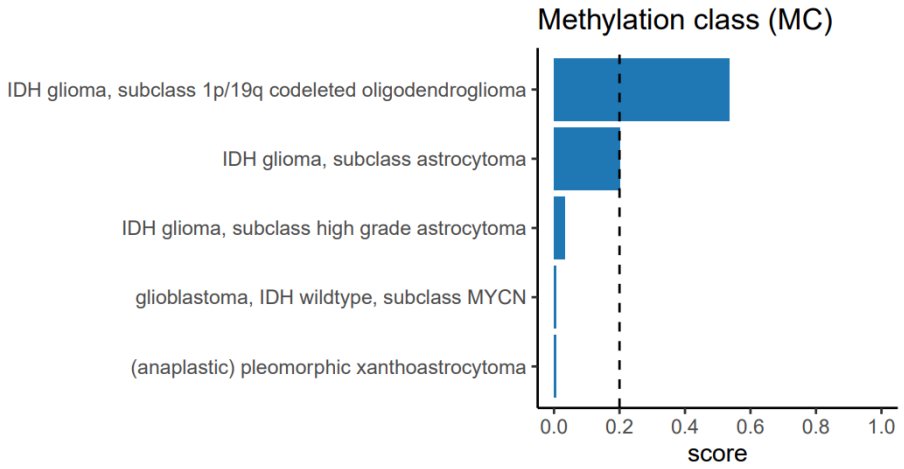


Oligodendroglioma 1p/19q deletion

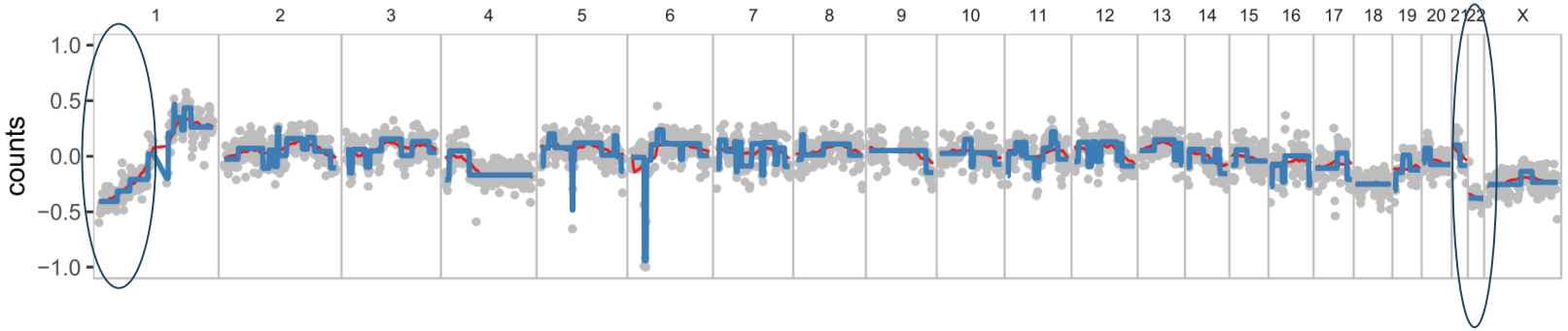
Methylation-based classification

Methylation-based classification is based on **111661** CpG sites (overlap of sites covered in this sample and the model). At the methylation class (MC) level, the sample has been classified as **IDH glioma, subclass 1p/19q codeleted oligodendroglioma**. This prediction has a confidence score of **0.537**. At the methylation class **family** (MCF) level, the sample has been classified as **glioma, IDH mutant**. The MCF prediction has a confidence score of **0.772**.

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.



Copy number profile

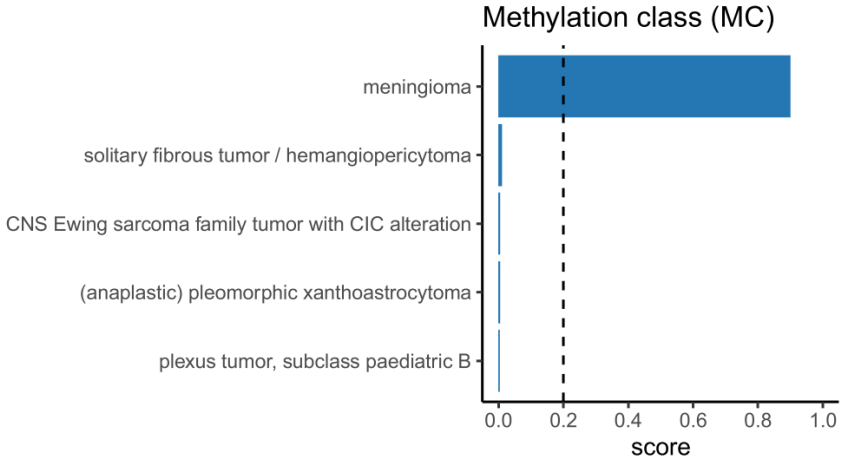


Meningeoma, 1p/22q loss
Atypical meningeoma,
WHO grade 2

Methylation-based classification

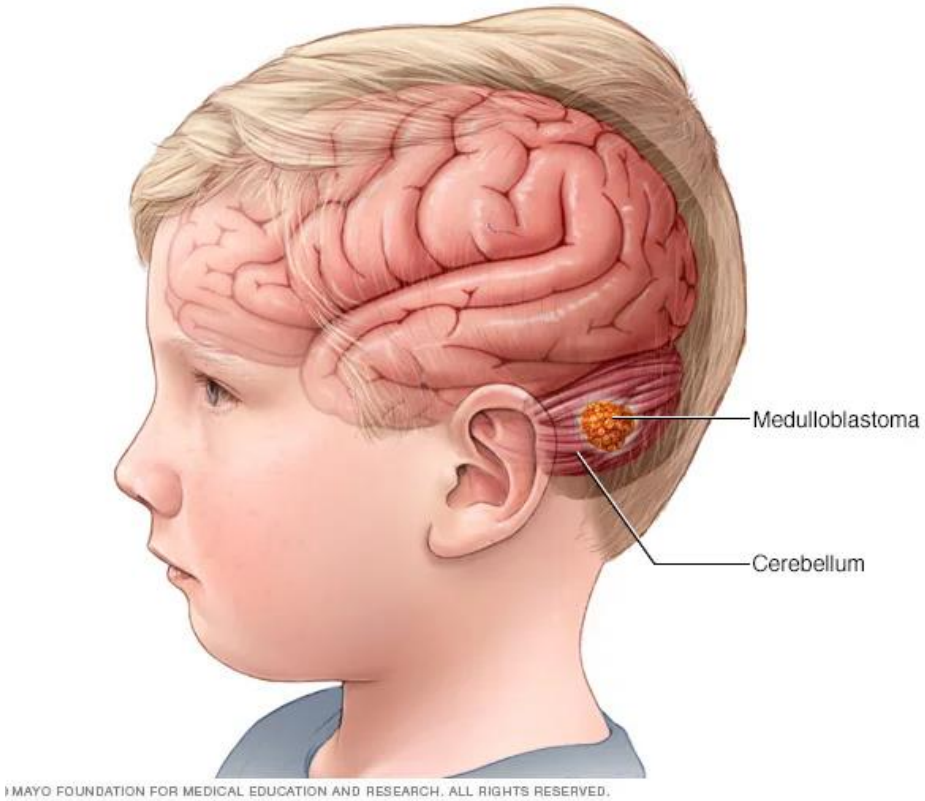
Methylation-based classification is based on **144053** CpG sites (overlap of sites covered in this sample and the model). At the methylation class (MC) level, the sample has been classified as **meningioma**. This prediction has a confidence score of **0.899**. At the methylation class **family** (MCF) level, the sample has been classified as **meningioma**. The MCF prediction has a confidence score of **0.899**.

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.

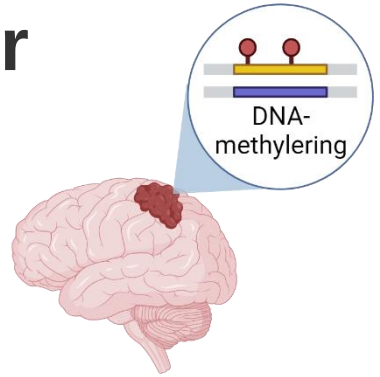


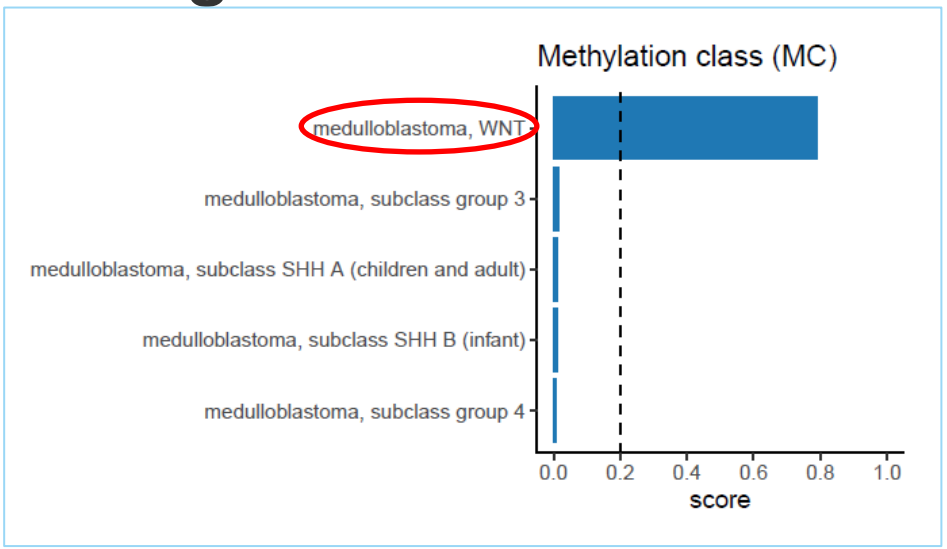
Patient case

- 3-year-old boy
- Tumour in 4th ventricle, hydrocephalus
- Frozen/smear: Small cell malignant tumour, most likely medulloblastoma.
- Medulloblastoma:
  Molecular subtypes
  - Wnt
  - SHH, p53wt, p53 mutated
  - Group 3
  - Group 4

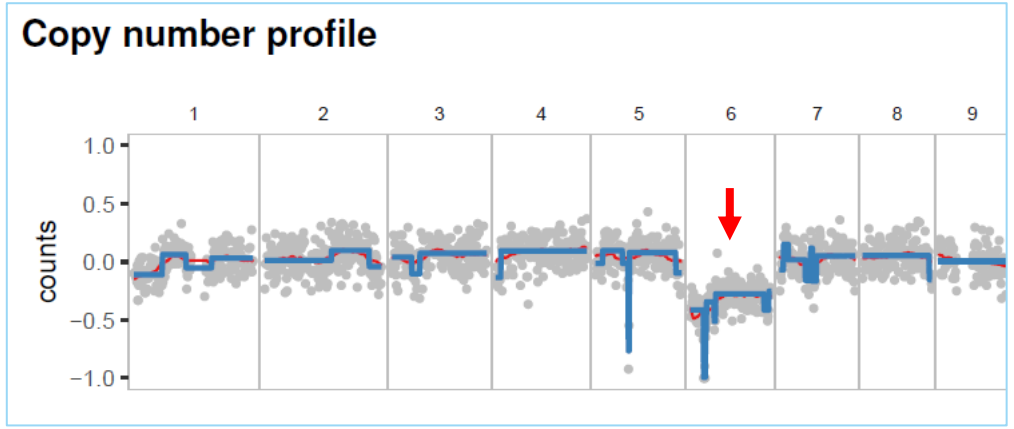


Example: Rapid diagnostics of childhood cancer



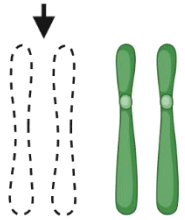


nanoDx
analysis



Match: Medulloblastoma
Subclass WNT-activated

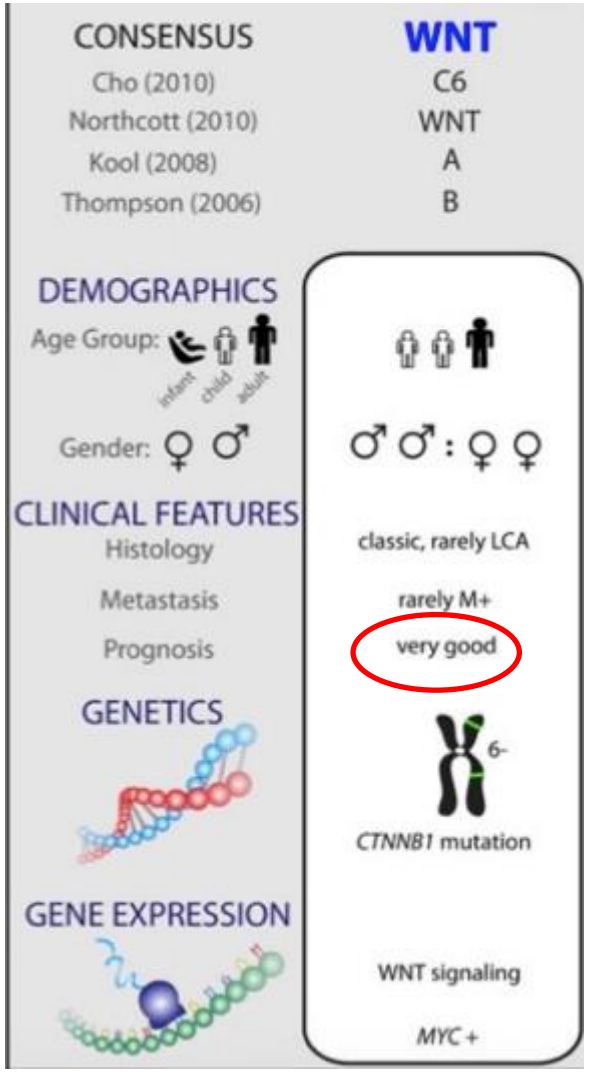
Subclass with best prognosis



Loss of chromosome 6
→ Typical for WNT

Patient case

- Operated Thursday
- Diagnosis ready Friday morning
- Diagnosis: Medulloblastoma, WNT activated (10%)
- Confirmed by EPIC analysis and *CTNNB1* mutation detected: c.98C>T, p.S33F (42%)
- Radiation and chemotherapy
- Survival rate 100%
- Intraoperative diagnostics?



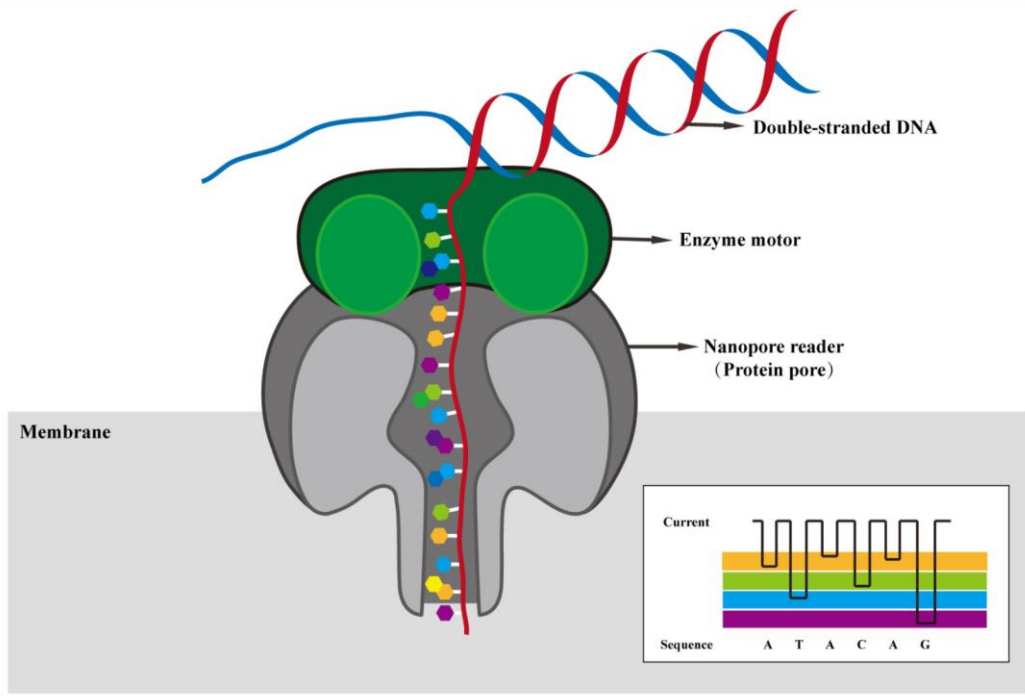
Use of NanoDx in daily diagnostics

- Routine diagnostics of gliomas – IDH mutation status and grading
  - → Reduction in IHC stainings (-7 per case)
  - → Reduction in NGS analyses for IDH status
- Routine diagnostics of meningiomas – grading according to CNV
  - → Reduction in IHC stainings (-2 per case)
  - → Improvement of grading (1p/22q and other chromosomal losses)
- Children
  - → Rapid diagnosis and start of treatment
- Rare forms of tumors
  - → Clues for further analyses

Conclusion, nanopore in our department

- Rapid and robust methylation-based classification in gliomas, meningiomas and pediatric CNS tumors.
- Accurate molecular diagnosis within 1-2 days
- Significantly reduction of IHC stainings
- Reduction in number of NGS- and Epic analyses
- Identifies CNVs useful for grading and classification
- Implementation was quick, despite lack of IT-/bioinformatic infrastructure

Nanopore, future perspectives



PromethION 2 Integrated "P2i"

Adaptive sequensing:

- Bed file with regions of interest
- "read until"
- DNA is ejected or sequensed

"All" relevant genetic information within 2-3 days, in a single analysis:

- Methylation
- CNV
- Gene fusions
- SNVs/mutations

Methylation:

- Intraoperative, real time
- Multiple classifiers

Oncomine Childhood Cancer Assay, mutations

- ABL1*, ABL2*", ALK*", ACVR1*, AKT1*, APC^, ARID1A^, ARID1B^, ASXL1*, ASXL2*, ATRX^, BRAF*", CALR*, CBL*, CCND1*", CCND3*, CCR5*, CDK4*", CDK6", CDKN2A^, CDKN2B^, CEBPA^, CHD7^, CIC*, CREBBP*, CRLF1^, CRLF2*, CSF1R*, CSF3R*, CTNNB1*, DAXX*, DDX3X^, DICER1^, DNMT3A*, EBF1^, EED^, EGFR*", EP300*, ERBB2*", ERBB3*", ERBB4*, ESR1*, EZH2*, FAS^, FASLG*, FBXW7*, FGFR1*", FGFR2*", FGFR3*", FGFR4", FLT3*, GATA1^, GATA2*, GATA3^, GLI1", GLI2", GNA11*, GNA13^, GNAQ*, H3F3A*, HDAC9*, HIST1H3B*, HRAS*, ID3^, IDH1*, IDH2*, IGF1R", IKZF1^, IL7R*, JAK1*", JAK2*", JAK3*", KDM4C*, KDM6A^, KDR*, KIT*", KMT2D^, KRAS*", MAP2K1*, MAP2K2*, MDM2", MDM4", MET*", MPL*, MSH6*, MTOR*, MYC", MYCN", MYOD1^, NCOR2*, NF1^, NF2^, NOTCH1*, NPM1*, NRAS*, NT5C2*, PAX5*, PDGFRA*", PDGFRB*, PHF6^, PIK3CA*", PIK3R1*, PPM1D*, PRPS1^, PSMB5^, PTCH1^, PTEN^, PTPN11*, RAF1*, RB1^, RET*, RHOA*, RUNX1^, SETBP1*, SETD2*, SH2B3*, SH2D1A*, SMARCA4^, SMARCB1^, SMO*, SOCS2^, STAT3*, STAT5B*, SUFU^, SUZ12^, TCF3^, TET2^, TP53^, TPMT*, TSC1^, TSC2^, USP7*, WHSC1^, WT1^, XIAP^ og ZMYM3* *: Hotspots. ^: Hele den kodende sekvens. ": CNV (gain)

Oncomine Comprehensive Assay v3, mutations

- AKT1*", AKT2*", AKT3*", ALK*", AR*", ARAF*, ARID1A^, ATM^, ATR^, ATRX^, AXL*", BAP1^, BRAF*", BRCA1^, BRCA2^, BTK*, CCND1*", CCND2", CCND3", CCNE1", CBL*, CDK2", CDK4*", CDK6*", CDK12^, CDKN1B^, CDKN2A^, CDKN2B^, CHEK1^, CHEK2*, CREBBP^, CSF1R*, CTNNB1*, DDR2*, EGFR*", ERBB2*", ERBB3*, ERBB4*, ERCC2*, ESR1*", EZH2*, FANCA^, FANCD2^, FANCI^, FGF3", FGF19", FGFR1*", FGFR2*", FGFR3*", FGFR4*", FLT3*", FOXL2*, FBXW7^, GATA2*, GNA11*, GNAQ*, GNAS*, H3F3A*, HIST1H3B*, HNF1A*, HRAS*, IDH1*, IDH2*, IGF1R", JAK1*, JAK2*, JAK3*, KDR*, KIT*", KNSTRN*, KRAS*", MAGOH*, MAP2K1*, MAP2K2*, MAP2K4*, MAPK1*, MAX*, MDM2", MDM4*", MED12*, MET*", MLH1^, MRE11^, MSH2^, MSH6^, MTOR*, MYC*", MYCL", MYCN*", MYD88*, NBN^, NF1^, NF2^, NFE2L2*, NOTCH1^, NOTCH2^, NOTCH3^, NRAS*, NTRK1*", NTRK2*", NTRK3*", PALB2^, PDGFRA*", PDGFRB*", PIK3CA*", PIK3CB*", PIK3R1^, PMS2^, POLE^, PPARG", PPP2R1A*, PTCH1^, PTEN^, PTPN11*, RAC1*, RAD50^, RAD51^, RAD51B^, RAD51C^, RAD51D^, RAF1*, RB1^, RET*, RHEB*, RHOA*, RICTOR", RNF43^, ROS1*, SETD2^, SF3B1*, SLX4^, SMAD4*, SMARCA4^, SMARCB1^, SMO*, SPOP*, SRC*, STAT3*, STK11^, TERT*", TOP1*, TP53^, TSC1^, TSC2^, U2AF1* og XPO1* *: Hotspots. ^: Hele den kodende sekvens. ": CNV (gain

Archer FUSIONPlex Sarcoma Expanded pane

- ALK*", BCOR*, BRAF*", CAMTA1*, CIC*, CSF1*, CTNNB1", EGFR*", EPC1*, ERG*, ESR1*, EWSR1*, FGFR1*", FGFR2*", FGFR3*", FOS*, FOSB*, FOXO1*, FUS*, GLI1*, HMGA2*, JAZF1*, MDM2*, MEAF6*, MET*, MGEA5*, MKL2*, MYOD1", NCOA1*, NCOA2*, NR4A3*, NTRK1*", NTRK2*", NTRK3*", NUTM1*, PAX3*, PDGFB*, PHF1*, PLAG1*, PRKCA*, PRKCB*, PRKCD*, RAF1*, RET*", ROS1*", SS18*, STAT6*, TAF15*, TCF12*, TFE3*, TFG*, USP6*, VGLL2*, YAP1* og YWHAE* og deres fusionspartnere (kendte som ukendte) *: Fusionsanalyse, ": Hotspot mutationsanalys

Archer FUSIONPlex Solid tumor panel

- AKT3*, ALK*, ARHGAP26*, AXL*, BRAF*", BRD3*, BRD4*, EGFR* (inkl. exon 2-7 skipping; EGFRvIII), ERG*, ESR1*, ETV1*, ETV4*, ETV5*, ETV6*, EWSR1*, FGFR1*, FGFR2*, FGFR3*, FGR*, INSR*, MAML2*, MAST1*, MAST2*, MET* (inkl. MET exon 14 skipping), MSMB*, MUSK*, MYB*, NOTCH1*, NOTCH2*, NRG1*, NTRK1*, NTRK2*, NTRK3*, NUMBL*, NUTM1*, PDGFRA*", PDGFRB*, PIK3CA*, PKN1*, PPARG*, PRKCA*, PRKCB*, RAF1*, RELA*, RET*, ROS1*, RSPO2*, RSPO3*, TERT*, TFE3*, TFEB*, THADA* og TMPRSS2* og deres fusionspartnere (kendte som ukendte) *Fusionsanalyse, ": Hotspot mutationsanalyse

Oncomine Comprehensive Assay Plus | Streamlined Workflow

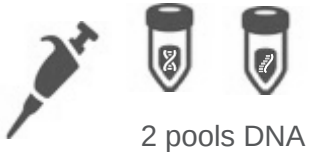
Low FFPE input successful sequencing and results from just 10 ng/pool FFPE

Fast turn-around time –results in **3-4 days**

1 **Minimal input**

10 ng/pool FFPE

2 **Library preparation**



2 pools DNA
2 pools RNA

3 **Multiplex sequencing**

Multiplex 4 samples

4 **Analysis & reporting**

Internally developed and optimized

Oncomine Comprehensive Assay Plus

Robust gene content and complex biomarkers detection

165 genes with recurrent hotspot mutations

333 genes with focal CNV gains or loss

227 genes with full-coding DNA sequence (CDS)

>1 mb Exonic footprint for Tumor Mutational Burden (TMB)

MSI-H/MSS Markers for Microsatellite Instability (MSI)

49 Fusion driver genes

MET exon skipping detection at DNA and RNA level

Cellularity (Tumor fraction) calculation

46 genes in Homologous Recombination Repair pathway

Loss of heterozygosity detection – gene level and sample level

Powerful bioinformatics solution for analyzing and visualizing **Mutational signatures**

Oncomine Comprehensive Assay Plus | Gene List

Hotspot only Genes* (n=57)

ACVR1	KLF4
ATP1A1	KNSTRN
BCR	MAP2K2
BMP5	MED12
BTK	MYOD1
CACNA1D	NSD2
CD79B	NT5C2
CSF1R	NTRK2
CTNNB1	NUP93
CUL1	PAX5
CYSLTR2	PIK3CD
DGCR8	PIK3CG
DROSHA	PTPRD
E2F1	RGS7
EPAS1	RHOA
FGF7	RPL10
FOXL2	SIX1
FOXO1	SIX2
GLI1	SNCAIP
GNA11	SOS1
GNAQ	SOX2
H2BC5 (HIST1H2BD)	SRSF2
H3C2 (HIST1H3B)	STAT5B
HIF1A	TAF1
HRAS	TGFBR1
IDH1	TRRAP
IL6ST	TSHR
IRF4	WAS
IRS4	

CNV Gain Genes* (n=19)

ABCB1
CTNND2
DDR1
EMSY
FGF19
FGF23
FGF3
FGF4
FGF9
FYN
GLI3
IGF1R
MCL1
MDM2
MYCL
RPS6KB1
RPTOR
YAP1
YES1

CNV gain and Hotspot Genes* (n=108)

ABL1	ERBB2	MAX	PTPN11
ABL2	ERBB3	MDM4	PXDNL
AKT1	ERBB4	MECOM	RAC1
AKT2	ESR1	MEF2B	RAF1
AKT3	EZH2	MET	RARA
ALK	FAM135B	MITF	RET
AR	FGFR1	MPL	RHEB
ARAF	FGFR2	MTOR	RICTOR
AURKA	FGFR3	MYC	RIT1
AURKC	FGFR4	MYCN	ROS1
AXL	FLT3	MYD88	SETBP1
BCL2	FLT4	NFE2L2	SF3B1
BCL2L12	FOXA1	NRAS	SLCO1B3
BCL6	GATA2	NTRK1	SMC1A
BRAF	GNAS	NTRK3	SMO
CARD11	H3-3A (H3F3A)	PCBP1	SPOP
CBL	H3-3B (H3F3B)	PDGFRA	SRC
CCND1	IDH2	PDGFRB	STAT3
CCND2	IKBKB	PIK3C2B	STAT6
CCND3	IL7R	PIK3CA	TERT
CCNE1	KDR	PIK3CB	TOP1
CDK4	KIT	PIK3R2	TPMT
CDK6	KLF5	PIM1	U2AF1
CHD4	KRAS	PLCG1	USP8
DDR2	MAGOH	PPP2R1A	XPO1
EGFR	MAP2K1	PPP6C	ZNF217
EIF1AX	MAPK1	PRKACA	ZNF429

Gene Fusions** (Inter- and Intra-genic) (n=49)

AKT1	NOTCH2
AKT2	NOTCH3
AKT3	NRG1
ALK	NTRK1
AR	NTRK2
BRAF	NTRK3
BRCA1	NUTM1
CDKN2A	PIK3CA
EGFR	PIK3CB
ERBB2	PPARG
ERBB4	PRKACA
ERG	PRKACB
ESR1	RAF1
ETV1	RARA
ETV4	RELA
ETV5	RET
FGFR1	ROS1
FGFR2	RSPO2
FGFR3	RSPO3
MAP3K8	STAT6
MET	TERT
MTAP	TFE3
MYB	TFEB
MYBL1	YAP1
NOTCH1	

Note: In parenthesis are shown gene symbols that have been updated since IR5.12 launch

Highlighted in red are genes recently reclassified as LOF.

*Gene content based on IR5.16 workflow- Feb 2021

Oncomine Comprehensive Assay Plus | Gene List, cont'd

CNV genes with CDS (n=206)

ABRAXAS1
ACVR1B
ACVR2A
ADAMTS12
ADAMTS2
AMER1
APC
ARHGAP35
ARID1A
ARID1B
ARID2
ARID5B
ASXL1
ASXL2
ATM*
ATR
ATRX
AXIN1
AXIN2
B2M
BAP1
BARD1*
BCOR
BLM*
BMPR2
BRCA1*
BRCA2*
BRIP1*
CASP8
CBFB
CD274
CD276
CDC73
CDH1
CDH10
CDK12*
CDKN1A
CDKN1B
CDKN2A
CDKN2B
CDKN2C
CHEK1*
CHEK2*
CIC
CREBBP
CSMD3
CTCF
CTLA4
CUL3
CUL4A
CUL4B
CYLD
CYP2C9
DAXX
DDX3X
DICER1
DNMT3A
DSC3
ELF3
ENO1
EP300
EPCAM
EPHA2
ERAP1
ERAP2
ERCC2
ERCC4
ERRFI1
ETV6
FANCA
FANCC
FANCD2
FANCE
FANCF
FANCG
FANCI
FANCL*
FANCM
FAT1
FBXW7
FUBP1
GATA3
GNA13
GPS2
HDAC2
HDAC9
HLA-A
HLA-B
HNF1A
INPP4B
JAK1
JAK2
JAK3
KDM5C
KDM6A
KEAP1
KMT2A
KMT2B
KMT2C
KMT2D
LARP4B
LATS1
LATS2
MAP2K4
MAP2K7
MAP3K1
MAP3K4
MAPK8
MEN1
MGA
MLH1
MLH3
MRE11
MSH2
MSH3
MSH6
MTAP
MUTYH
NBN*
NCOR1
NF1
NF2
NOTCH1
NOTCH2
NOTCH3
NOTCH4
PALB2*
PARP1
PARP2
PARP3
PARP4
PBRM1
PDCD1
PDCD1LG2
PDIA3
PGD
PHF6
PIK3R1
PMS1
PMS2
POLD1*
POLE*
POT1
PPM1D
PPP2R2A*
PRDM1
PRDM9
PRKAR1A
PTCH1
PTEN*
PTPRT
RAD50
RAD51
RAD51B*
RAD51C*
RAD51D*
RAD52
RAD54L*
RASA1
RASA2
RB1
RBM10
RECQL4
RNASEH2A
RNASEH2B
RNF43
RPA1
RUNX1
SDHA
SDHB
SDHD
SETD2
SLX4
SMAD2
SMAD4
SMARCA4
SMARCB1
SOX9
SPEN
STAG2
STK11
SUFU
TAP1
TAP2
TBX3
TCF7L2
TET2
TGFBR2
TNFAIP3
TNFRSF14
TP53*
TP63
TPP2
TSC1
TSC2
USP9X
VHL
WT1
XRCC2
XRCC3
ZFHX3
ZMYM3
ZRSR2

CDS Only Genes (n=21)

CALR
CIITA
CYP2D6
ERCC5
FAS
ID3
KLHL13
MTUS2
PSMB10
PSMB8
PSMB9
RNASEH2C
RPL22
RPL5
RUNX1T1
SDHC
SOCS1
STAT1
TMEM132D
UGT1A1
ZBTB20

TMB only genes** (n=90)

A1CF
ACSM2B
ADAM18
ANO4
ARMC4
AURKB
BRINP3
C6
C8A
C8B
CANX
CASR
CD163
CNTN6
CNTNAP4
CNTNAP5
COL11A1
DCAF4L2
DCDC1
GALNT17
GPR158
GRID2
H1-4 (HIST1H1E)
HCN1
HLA-C
KCND2
KCNH7
KCNJ5
KEL
KIR3DL1
KRTAP2-1
KRTAP6-2
LRRC7
MARCO
NLRC5
NOL4
NRXN1
NYAP2
OR10G8
OR2G6
OR2L13
OR2L2
OR2L8
OR2M3
OR2T3
OR2T33
OR2T4
OR2W3
OR4A15
OR4C15
OR4C6
OR4M1
OR4M2
OR5D18
OR5F1
OR5L1
OR5L2
OR6F1
OR8H2
OR8I2
OR8U1
ORC4
PAK5
PCDH17
PDE1A
PDE1C
PLXDC2
POM121L12
PPFIA2
RBP3
REG1A
REG1B
REG3A
REG3G
RPTN
RUNDC3B
SH3RF2
SLC15A2
SLC8A1
SYT10
SYT16
TAPBP
TOP2A
TPTE
TRHDE
TRIM48
TRIM51
ZIM3
ZNF479
ZNF536

Note: In parenthesis are shown gene symbols that have been updated since IR5.12 launch

*Homologous recombination repair genes enabled for gene-level LOH
** All genes used for TMB, but these genes are only used in TMB analys
Gene content based on IR5.16 workflow- Feb 2021

Workflow

- Nanopore på alle gliomer, meningeomer, og pædiatriske tumorer
- IHC analyser hvis nødvendigt
- NGS analyser med hensyn til mutationer og fusioner på alle pædiatriske cases
- Epic methyleringsanalyse på embryonale tumorer og mærkelige cases som ikke er inkluderet i v11.b4
- Intraoperativ nanopore for selekterede cases; barn hvor der skal laves intraoperativ MRI.
- Medulloblastomer; 3 af 4 korrekt molekylær subtype (Epic gold standard) efter 20 minutters sekventering og alle indenfor 40 minutters sekventering.

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