Epigenetic Alterations in Cancer:
current applications in diagnostics

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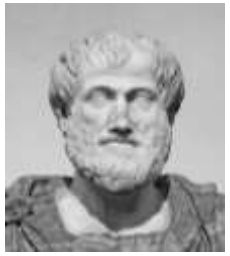
Molecular Pathology course, 20/05/2022

Overview

- Introduction
- Epigenetic mechanisms
- Epigenetics in cancer
  - Histone modification
  - DNA methylation
    - MGMT promoter methylation
    - Genome-wide methylation
- Conclusion

Epigenetics



Aristotle, 384-322 BC:

"… Epigenesis … development of individual organic form from unformed"

Conrad Waddington, 1942:

"… is the branch of biology which studies the causal interactions between genes and their products, which bring the phenotype into being"

Arthur Riggs, 1996:

"… is the study of mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes in DNA sequence"

Epigenetics

- Comes from the Greek ‘epi’: over or upon
- ‘Marks’ **around the DNA** that can turn genes on or off
  - ‘Marks’ are inherited and yet also reversible
- A layer on top of DNA that exerts an additional control over it

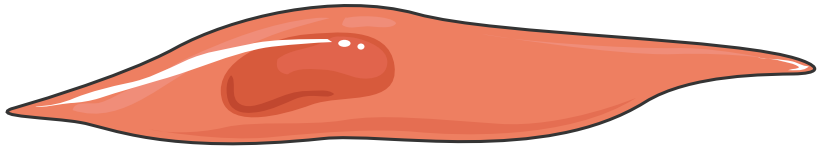
Epigenetics

All cells in the human body have the same DNA

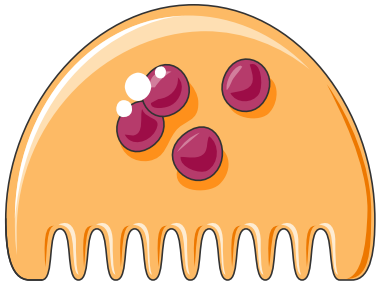
→ how come they have distinct functions?

Unique repertoire of gene expression

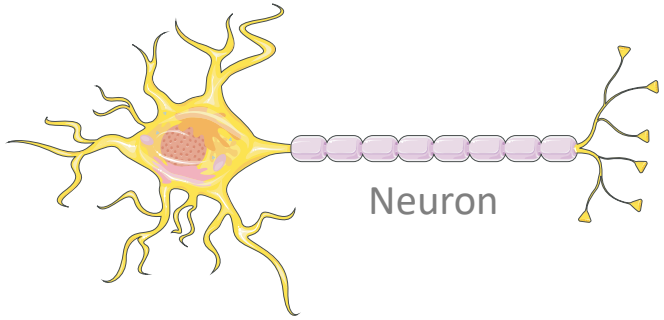
Gene expression is regulated by epigenetic modifications



Smooth muscle fiber



Osteoclast



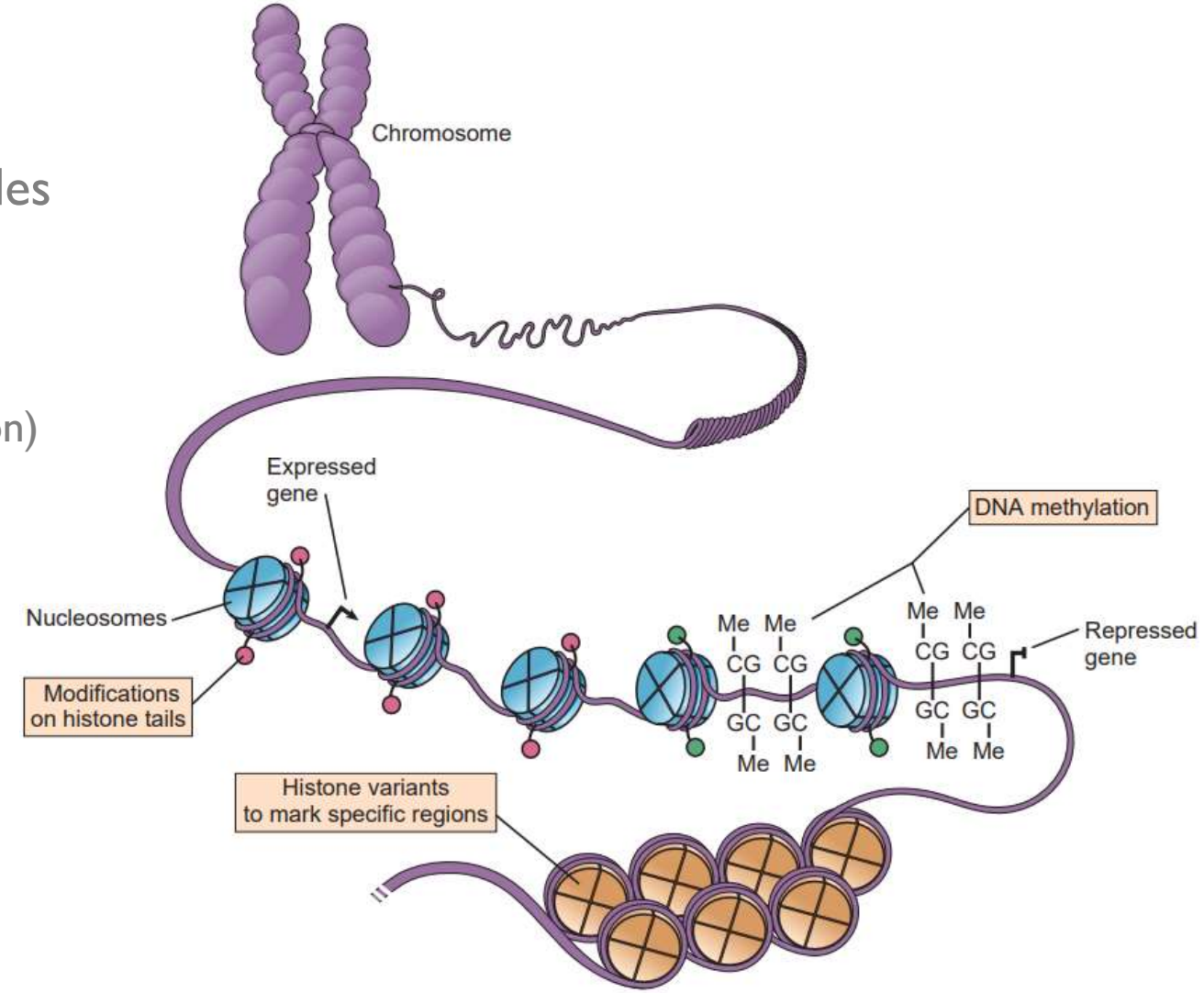
Neuron

Three major epigenetic mechanisms

1. DNA methylation at CpG dinucleotides
   → associated with gene repression

2. Modifications on histone tails
   (e.g. methylation, acetylation, phosphorylation)
   → associated with either gene expression or repression

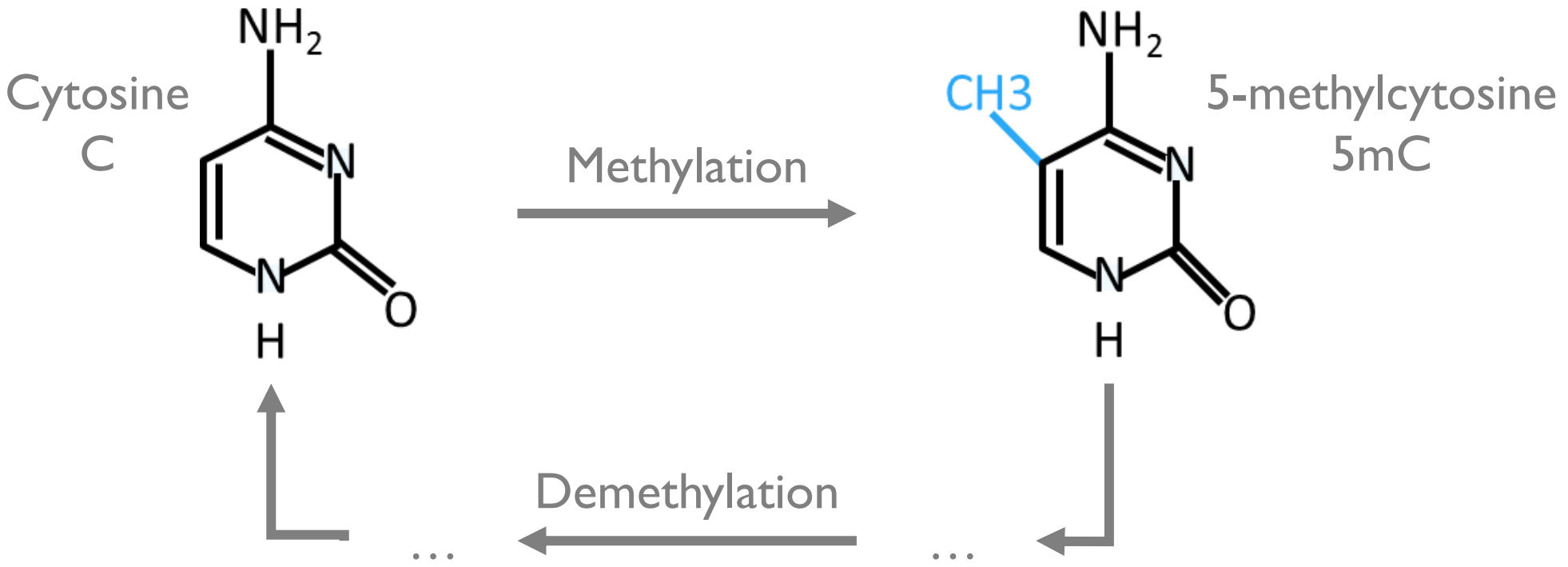
3. Histone variants
   → associated with specific functions required for chromosome stability and genome integrity



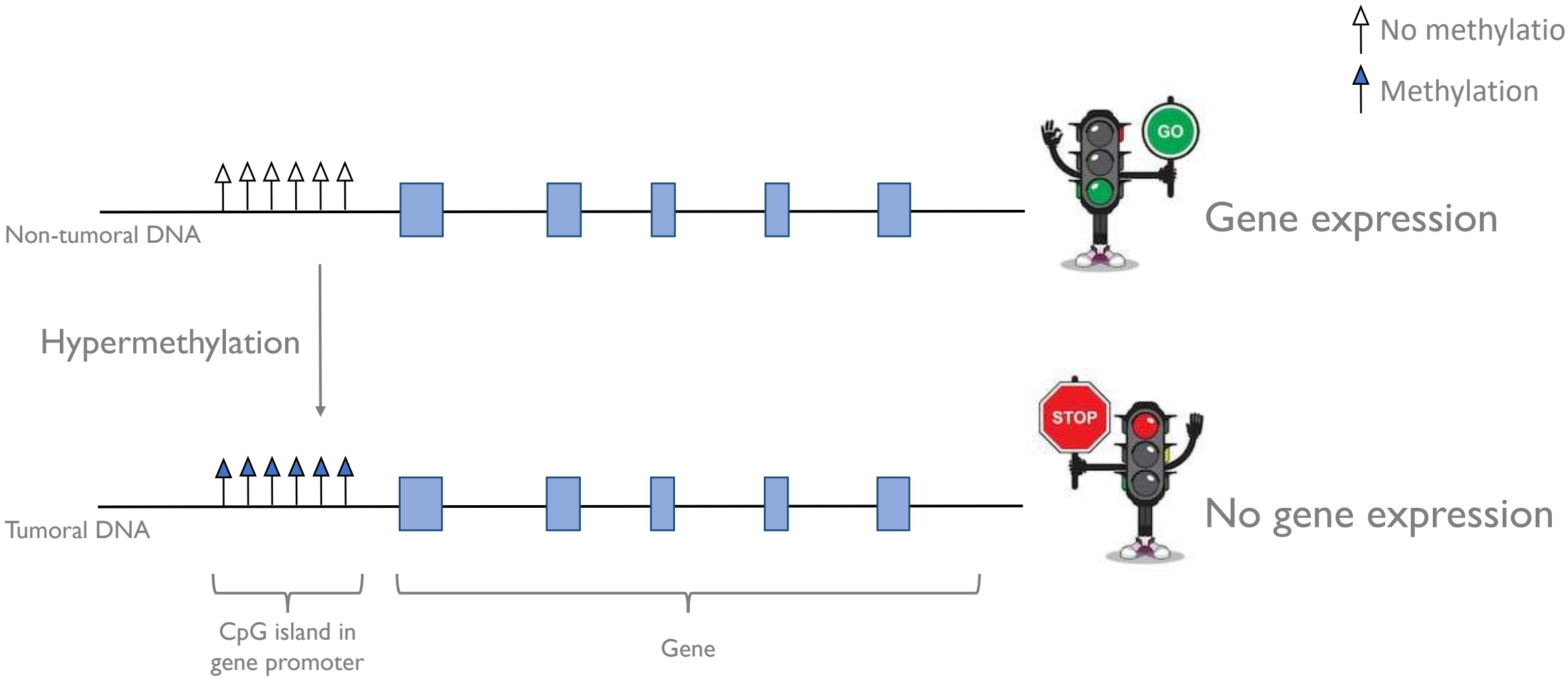
Thompson & Thompson Genetics in Medicine. Eighth edition. ISBN 978-1-4377-0696-3

DNA methylation

- 'Marks' **around the DNA** that can turn genes on or off
  - For example: methyl groups that bind to the DNA



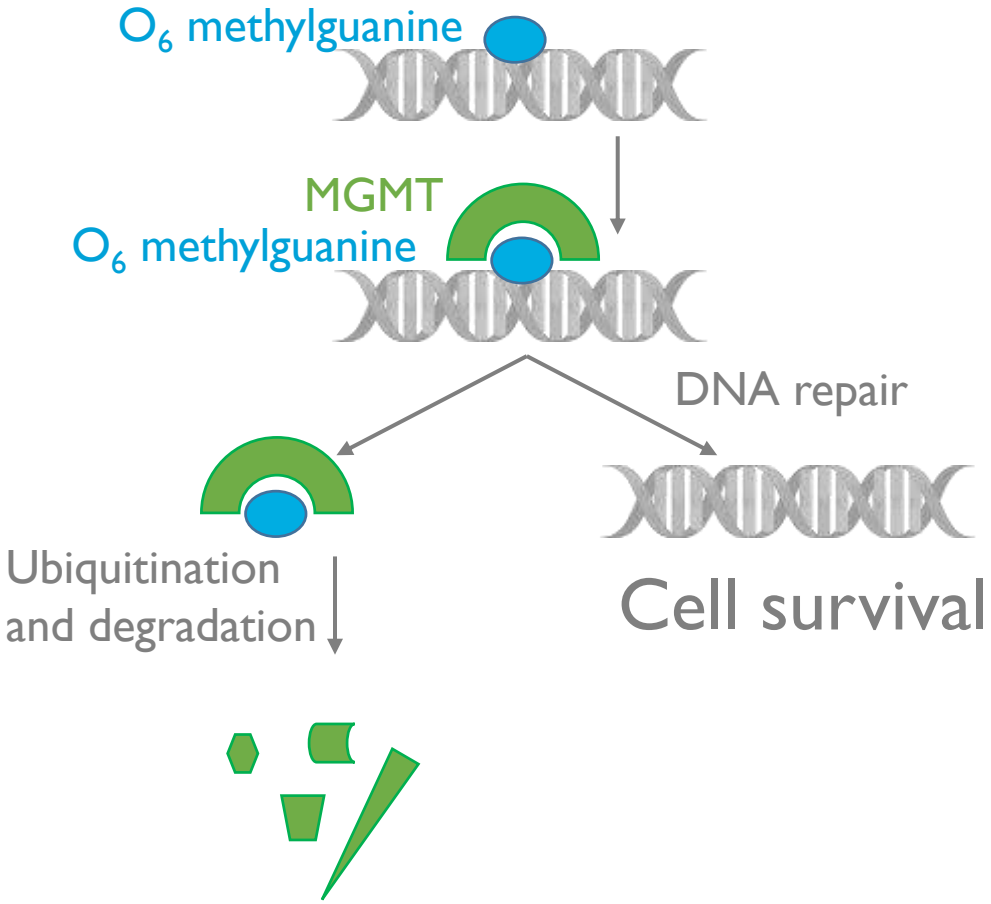
DNA methylation



Based on Pfeifer. Int J Mol Sci 2018;19(4):1166.

MGMT - function

- O_6 methyl guanine DNA methyltransferase
- Enzyme that repairs DNA
- Removes methyl-groups from O_6 site of guanine

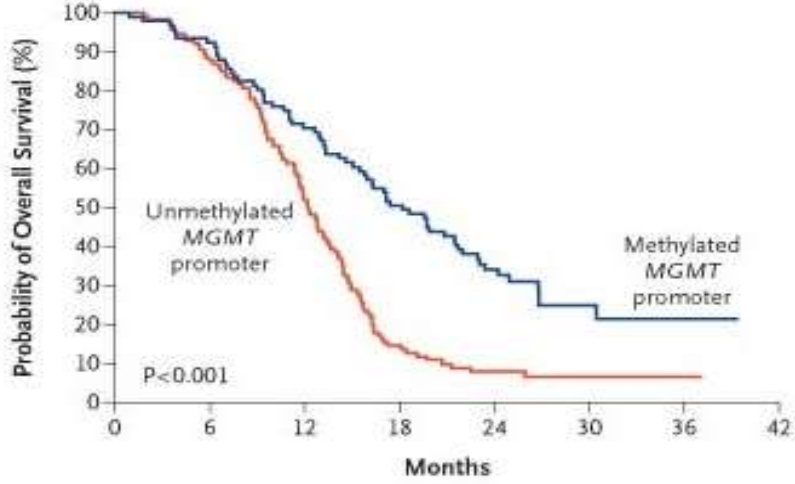


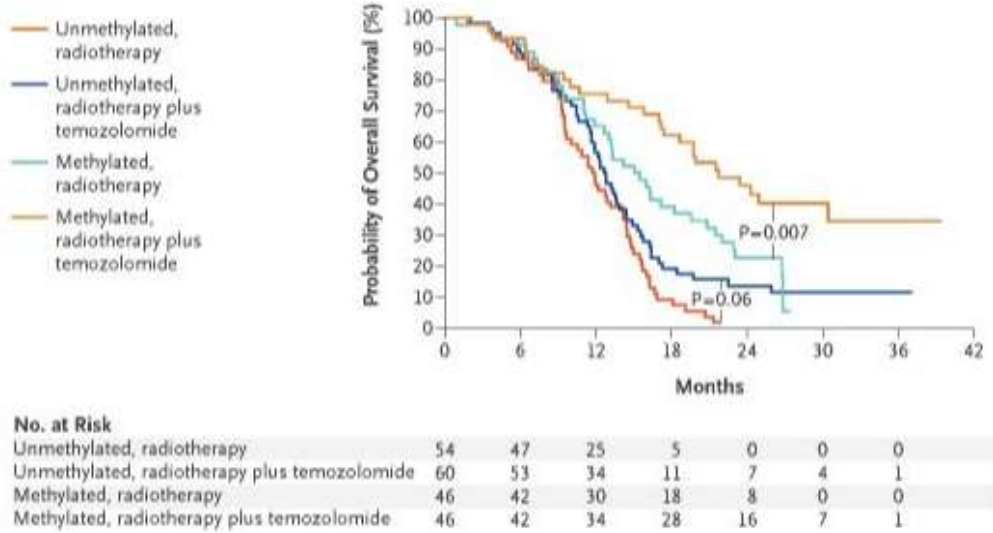
MGMT promoter methylation

- IDH-wildtype glioblastoma
  - 40-50% of patients have methylation of MGMT promoter
  - No active transcription of MGMT transcript
  - No DNA repair
  - DNA base mismatch and cross-linking
  - Activation of apoptosis

MGMT promoter methylation

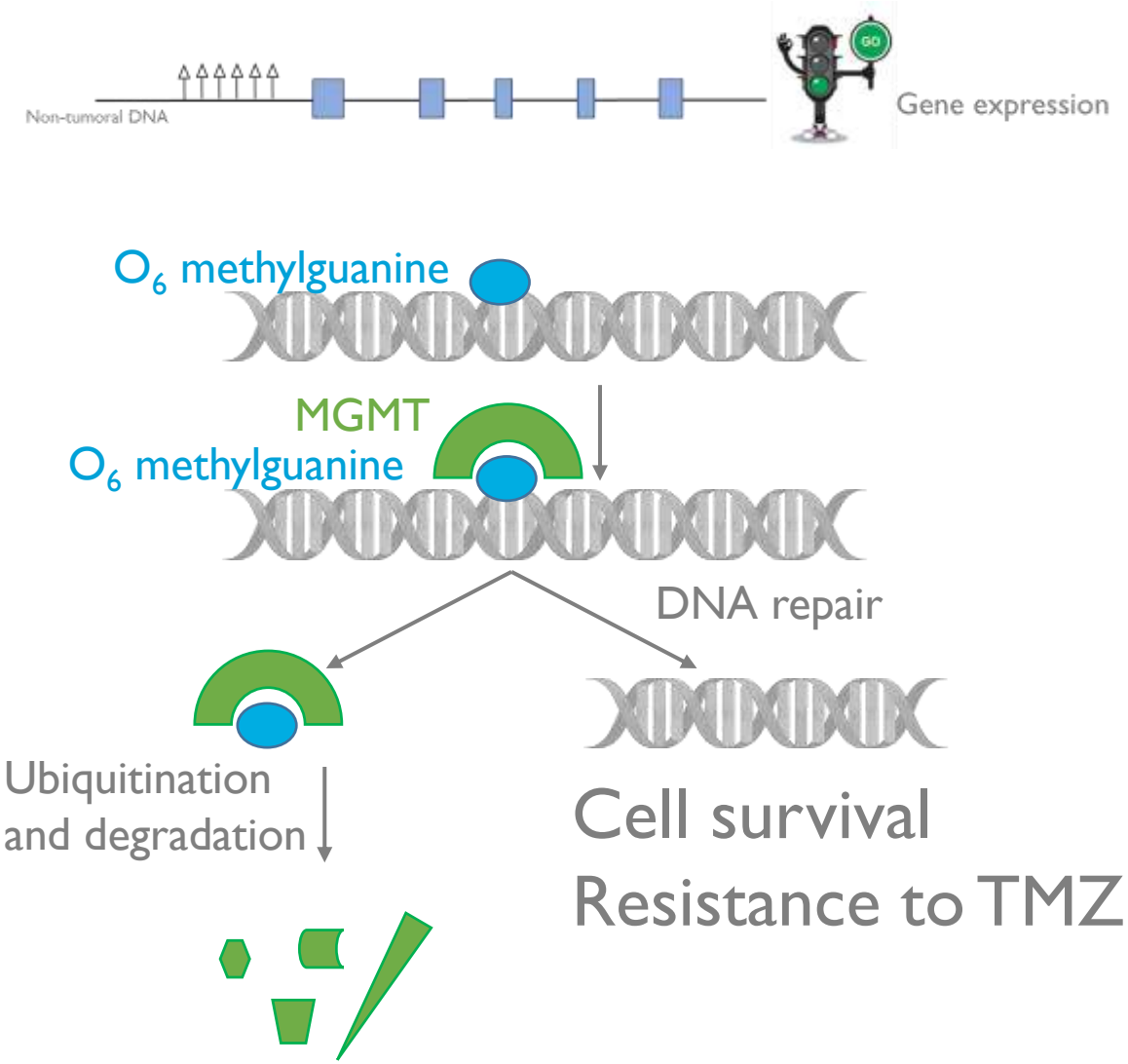
- Patients with glioblastoma, IDH-wildtype and MGMT promoter methylation have
  - Better prognosis (OS) than patients without MGMT promoter methylation
  - Better response to TMZ (temozolomide); alkylating chemotherapeutic agent that adds alkyl groups to DNA

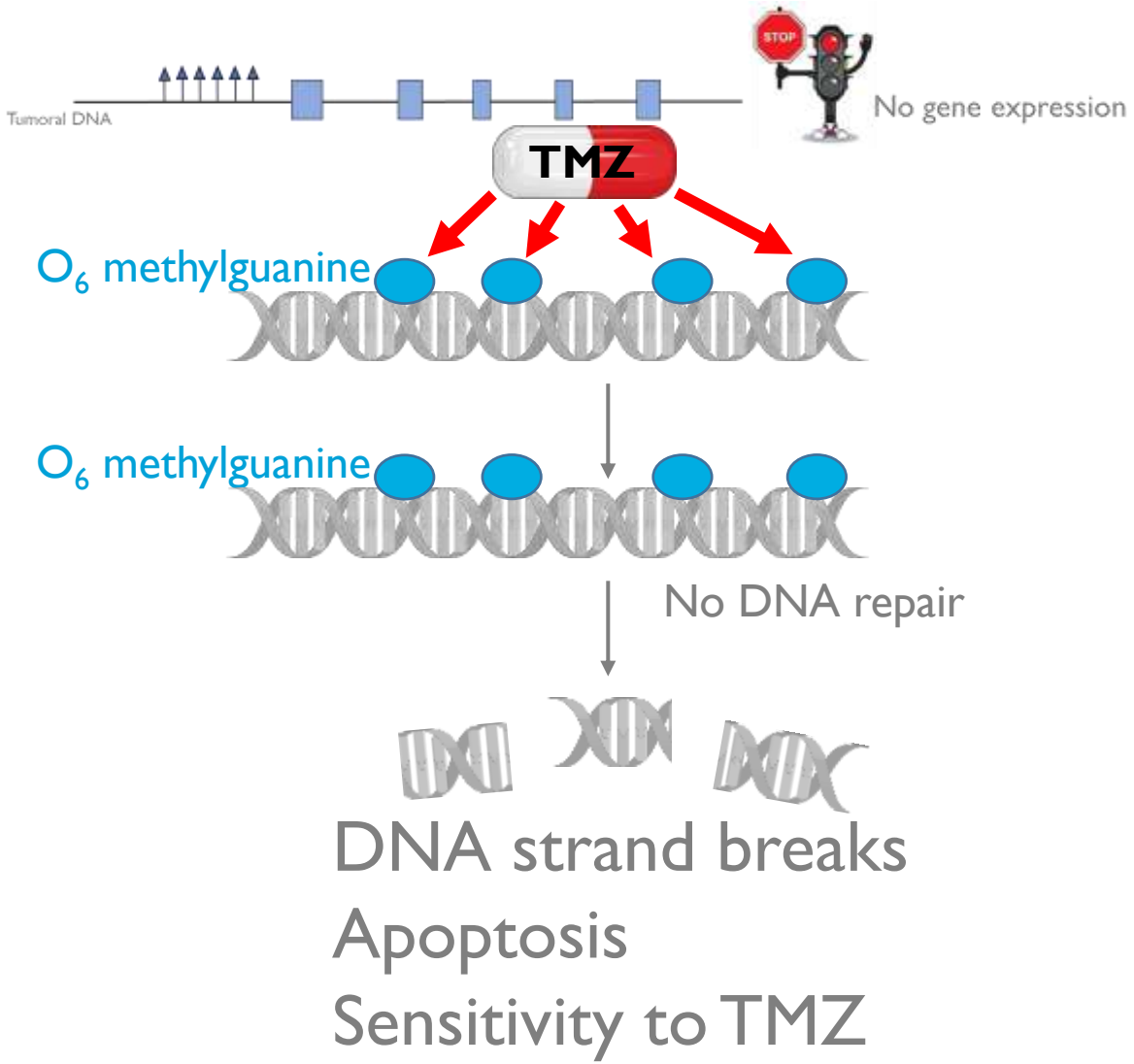




Hegi *et al.* N Engl J Med 2005;352(10):997-1003.

MGMT promoter methylation





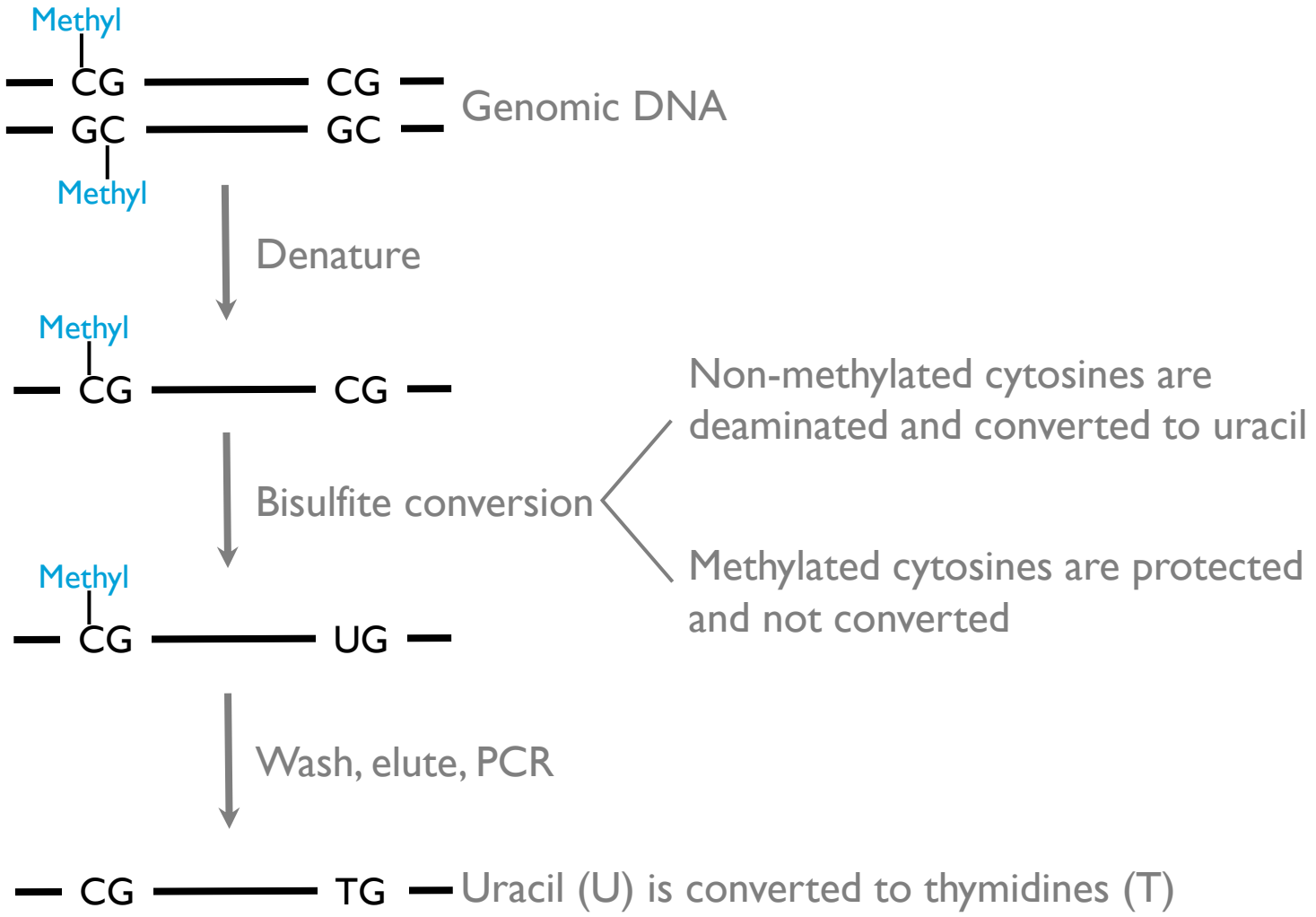
MGMT promoter methylation - detection

MSP	Methylation-specific PCR
qMSP	Quantitative real-time MSP
PSQ	Pyrosequencing
MS-MLPA	Methylation-specific Multiplex Ligation-dependent Probe Amplification
PCR with HRM	PCR with High-Resolution Melting
COLD-PCR	Co-amplification at Lower Denaturation temperature
Beadchip array	

MGMT – qMSP

1) Bisulfite conversion

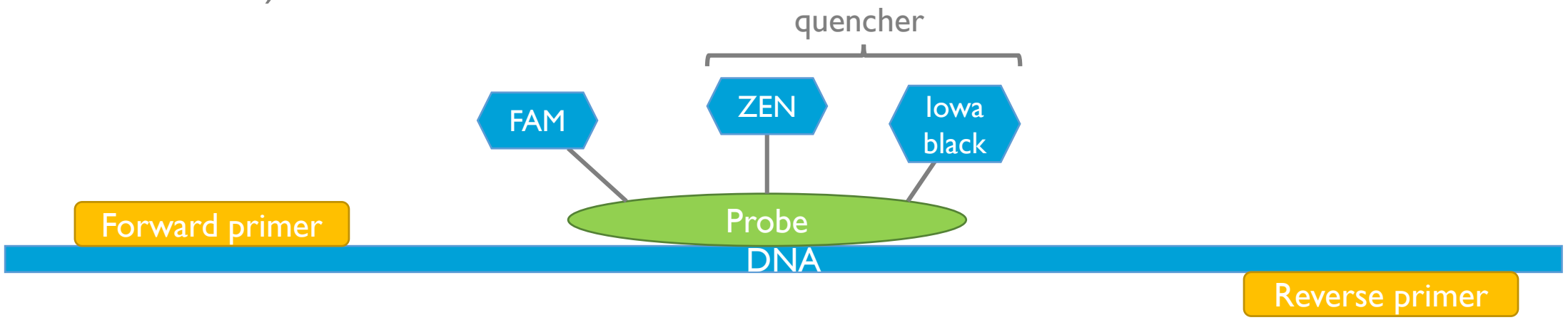
<u>Goal</u>:
convert genomic DNA to distinguish methylated from non-methylated cytosines



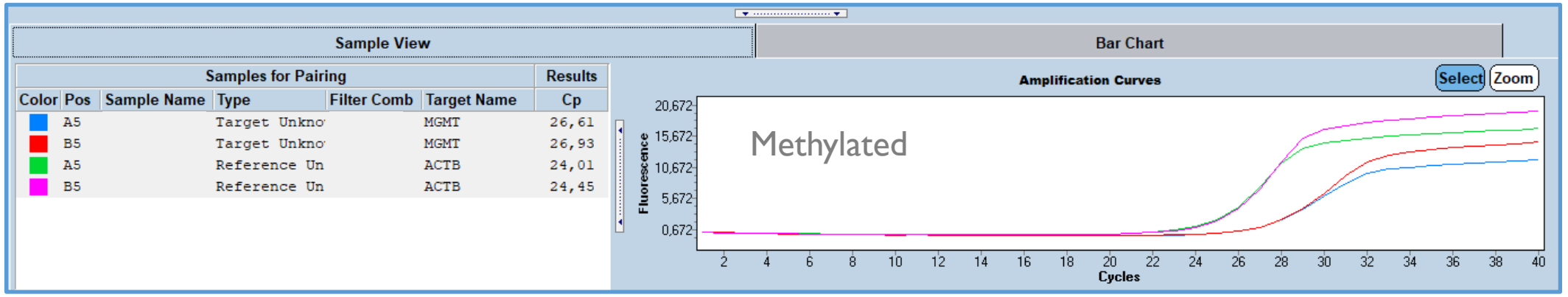
MGMT – qMSP

2) Real-time methylation specific PCR

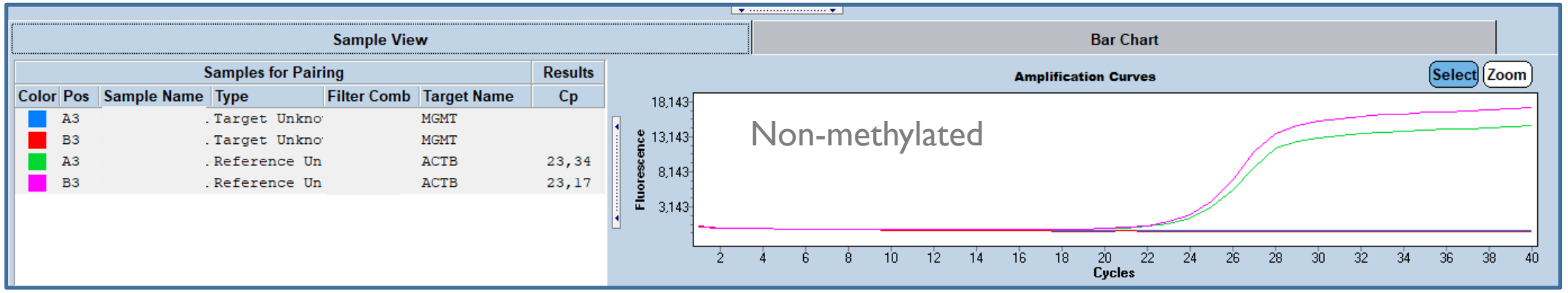
- 2 primers and 1 probe complementary to methylated MGMT sequence
- Second set of primers+probe complementary to β-actin (internal reference)



MGMT – qMSP output



β-actin
MGMT



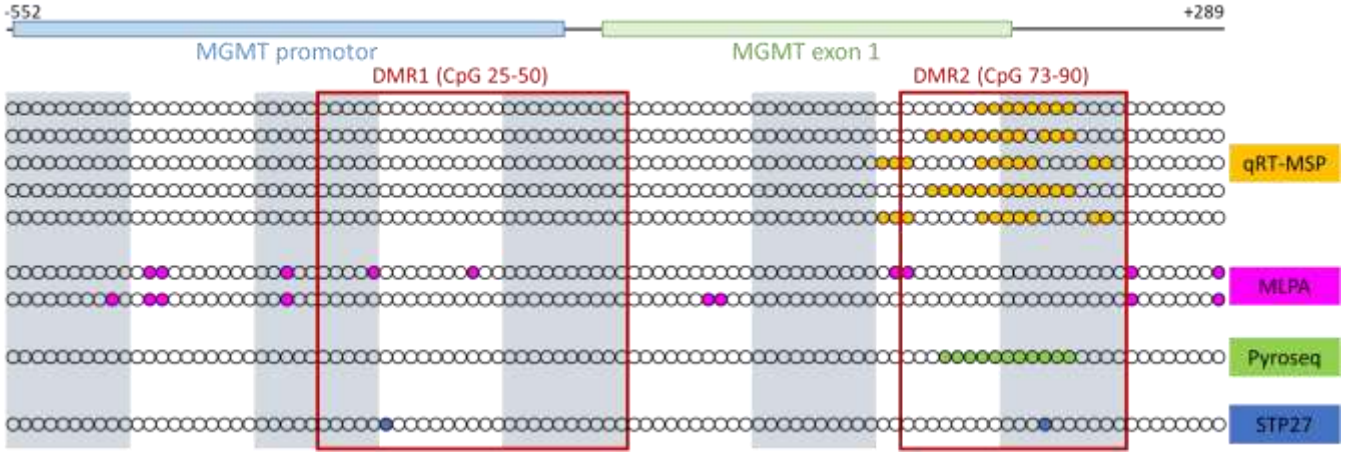
β-actin

MGMT

MGMT – remaining questions

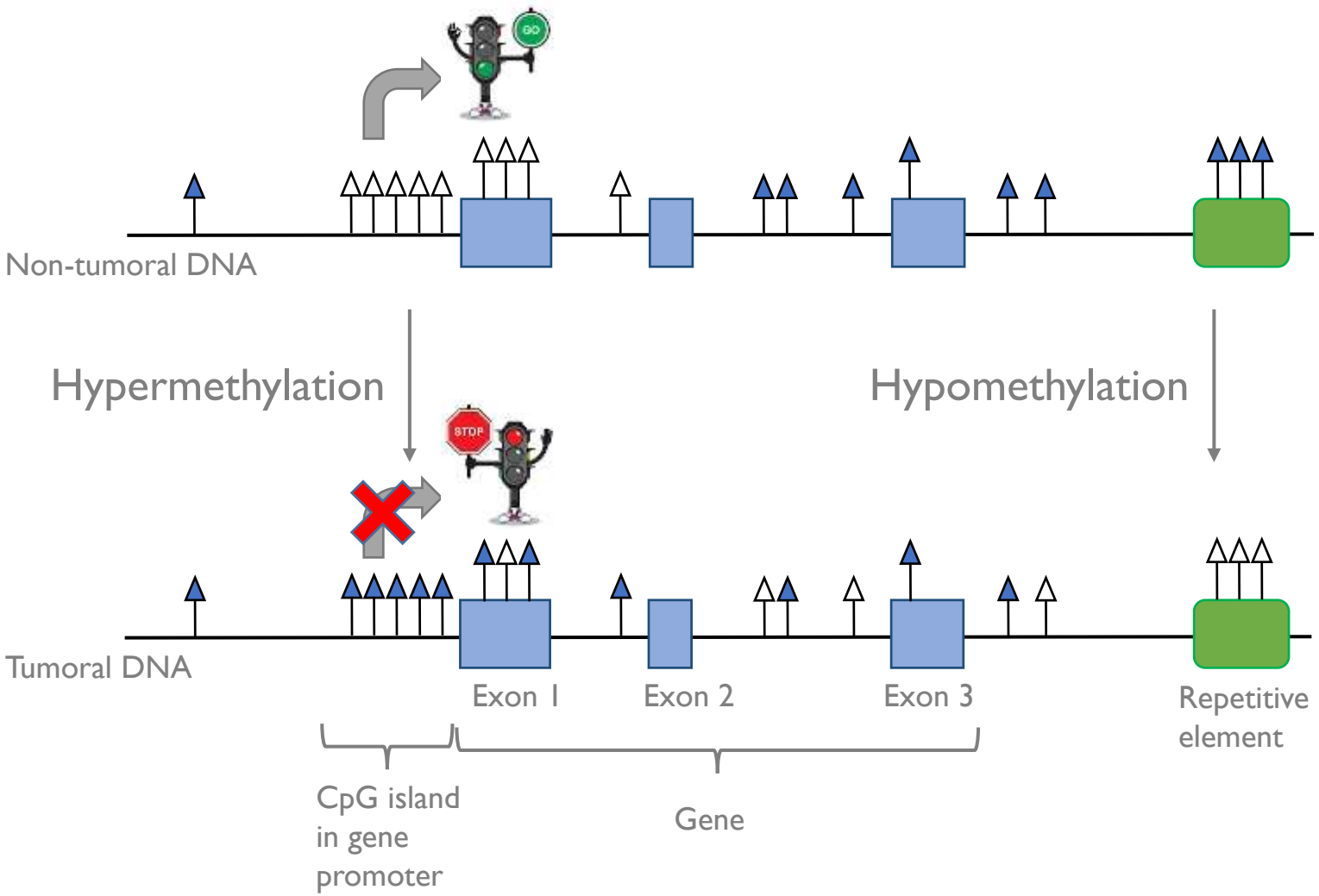
No internationally accepted consensus on

- the most appropriate diagnostic method
- which CpG sites to test (~ correlation with prognosis?)



- cutoff for categorizing methylation status

From promoter methylation to genome-wide methylation



- Hypermethylation:
  - Often in gene promoters which renders the gene inactive
- Hypomethylation
  - Genome-wide
  - Often in repetitive regions

No methylation

Methylation

Based on Pfeifer. Int J Mol Sci 2018;19(4):1166.

From promoter methylation to genome-wide methylation

ARTICLE

2018

doi:10.1038/nature26000

DNA methylation–based classification of central nervous system tumours

A list of authors and their affiliations appears in the online version of the paper.

Accurate pathological diagnosis is crucial for optimal management of patients with cancer. For the approximately 100 known tumour types of the central nervous system, standardization of the diagnostic process has been shown to be particularly challenging—with substantial inter-observer variability in the histopathological diagnosis of many tumour types. Here we present a comprehensive approach for the DNA methylation-based classification of central nervous system tumours across all entities and age groups, and demonstrate its application in a routine diagnostic setting. We show that the availability of this method may have a substantial impact on diagnostic precision compared to standard methods, resulting in a change of diagnosis in up to 12% of prospective cases. For broader accessibility, we have designed a free online classifier tool, the use of which does not require any additional onsite data processing. Our results provide a blueprint for the generation of machine-learning-based tumour classifiers across other cancer entities, with the potential to fundamentally transform tumour pathology.

Acta Neuropathologica (2018) 136:181–210
https://doi.org/10.1007/s00401-018-1879-y

ORIGINAL PAPER

CrossMark

Practical implementation of DNA methylation and copy-number-based CNS tumor diagnostics: the Heidelberg experience

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nature COMMUNICATIONS

ARTICLE

2021

https://doi.org/10.1038/s41467-020-20603-4 OPEN

Sarcoma classification by DNA methylation profiling

Sarcomas are malignant soft tissue and bone tumours affecting adults, adolescents and children. They represent a morphologically heterogeneous class of tumours and some entities lack defining histopathological features. Therefore, the diagnosis of sarcomas is burdened with a high inter-observer variability and misclassification rate. Here, we demonstrate classification of soft tissue and bone tumours using a machine learning classifier algorithm based on array-generated DNA methylation data. This sarcoma classifier is trained using a dataset of 1077 methylation profiles from comprehensively pre-characterized cases comprising 62 tumour methylation classes constituting a broad range of soft tissue and bone sarcoma subtypes across the entire age spectrum. The performance is validated in a cohort of 428 sarcomatous tumours, of which 322 cases were classified by the sarcoma classifier. Our results demonstrate the potential of the DNA methylation-based sarcoma classification for research and future diagnostic applications.

Received: 14 January 2022 | Revised: 16 March 2022 | Accepted: 18 March 2022
DOI: 10.1002/gcc.23041

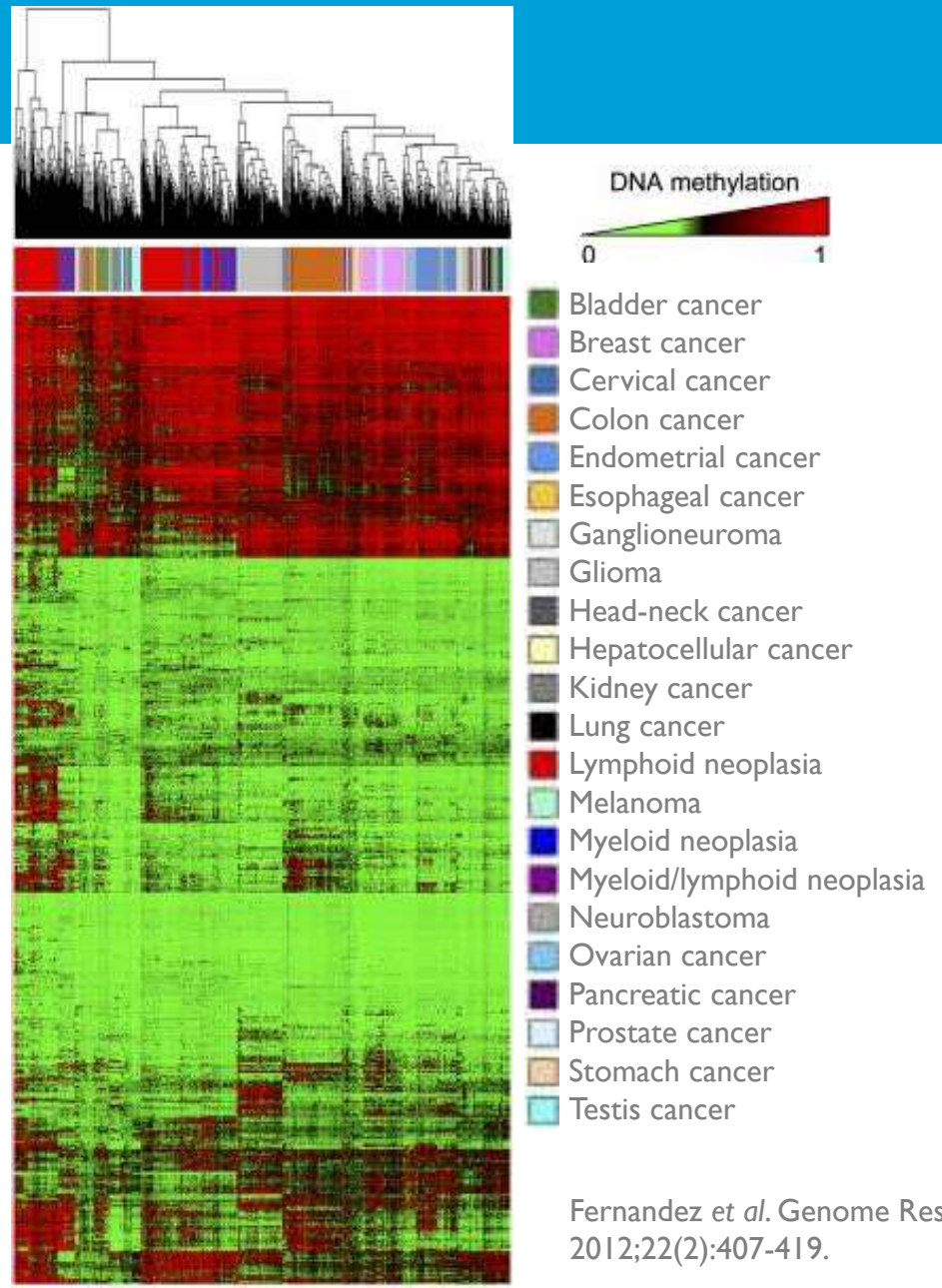
REVIEW ARTICLE

Methylation classifiers: Brain tumors, sarcomas, and what's next

Christian Koelsche[1] | Andreas von Deimling[2,3]

Cancer methylome

- Genome-wide DNA methylation pattern in cancer
- Represents both
  - The cell of origin
  - Somatically acquired DNA methylation changes
- Different tumor types have different DNA methylation profiles



Fernandez *et al.* Genome Res 2012;22(2):407-419.

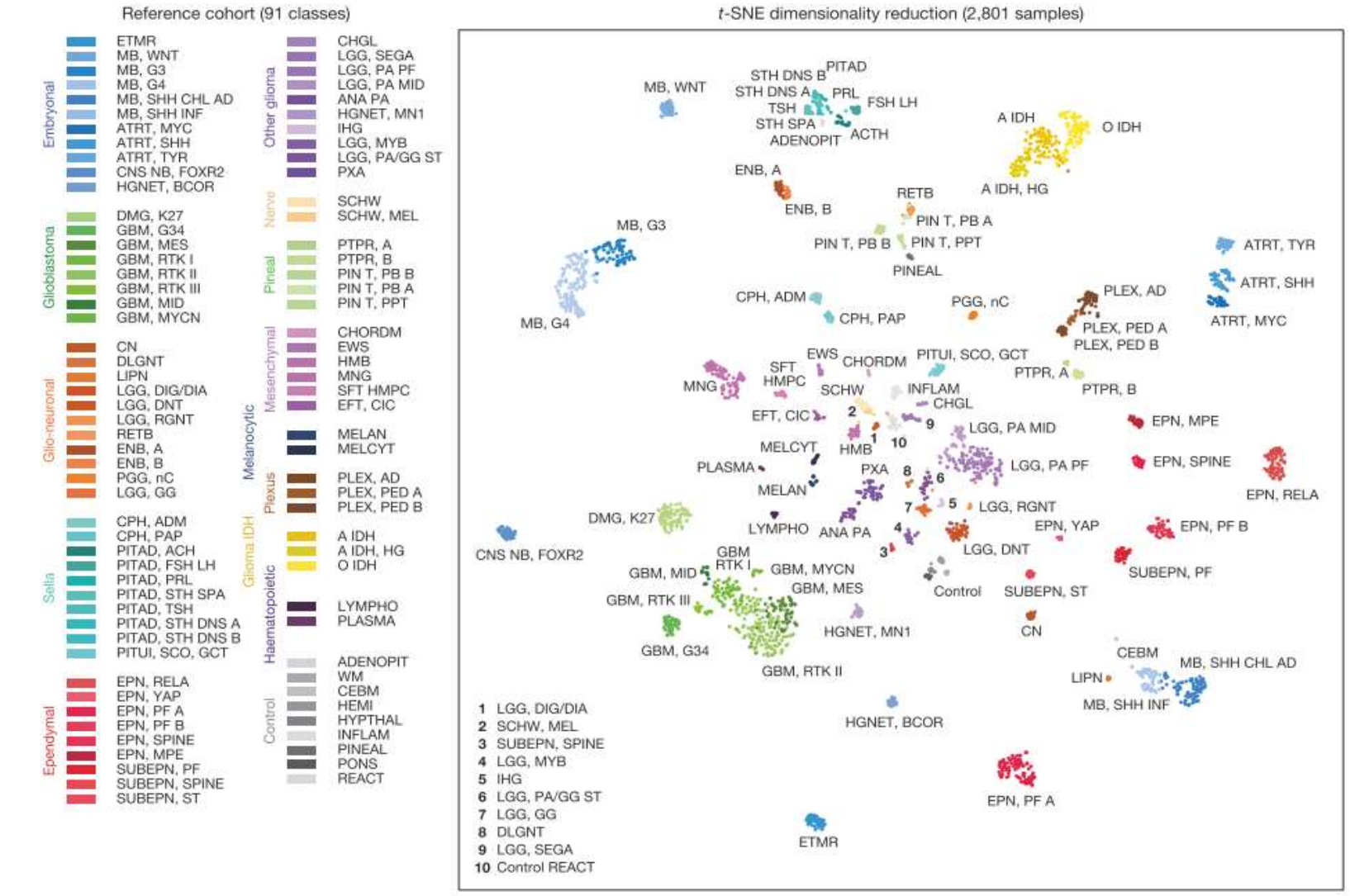
WHO classification of tumours of the central nervous system

- Gliomas, glioneuronal tumors, and neuronal tumors
  - Adult-type diffuse gliomas
    - Astrocytoma, IDH-mutant
    - Oligodendroglioma, IDH-mutant, and 1p/19q-codeleted
    - Glioblastoma, IDH-wildtype
  - Pediatric-type diffuse low-grade gliomas
    - Diffuse astrocytoma, *MYB*- or *MYBL1*-altered
    - Angiocentric glioma
    - Polymorphous low-grade neuroepithelial tumor of the young
    - Diffuse low-grade glioma, MAPK pathway-altered
  - Pediatric-type diffuse high-grade gliomas
    - Diffuse midline glioma, H3 K27-altered
    - Diffuse hemispheric glioma, H3 G34-mutant
    - Diffuse pediatric-type high-grade glioma, H3-wildtype and IDH-wildtype
    - Infant-type hemispheric glioma
  - Circumscribed astrocytic gliomas
    - Pilocytic astrocytoma
    - High-grade astrocytoma with piloid features
    - Pleomorphic xanthoastrocytoma
    - Subependymal giant cell astrocytoma
    - Chordoid glioma
    - Astroblastoma, *MN1*-altered
  - Glioneuronal and neuronal tumors
    - Ganglioglioma
    - Desmoplastic infar
    - Dysembryoplastic
    - *Diffuse glioneurona* *and nuclear clusters*
    - Papillary glioneurona
    - Rosette-forming glio tumor
    - Myxoid glioneuronal tumor
    - Diffuse leptomeningeal glioneuronal tumor
    - Gangliocytoma
    - Multinodular and vacuolating neuronal tumor
    - Dysplastic cerebellar gangliocytoma (Lhermitte-Duclos disease)
    - Central neurocytoma
    - Extraventricular neurocytoma
    - Cerebellar liponeurocytoma
  - Ependymal tumors
    - Supratentorial ependymoma
    - Supratentorial ependymoma, *ZFTA* fusion-positive
    - Supratentorial ependymoma, *YAP1* fusion-positive
    - Posterior fossa ependymoma
    - Posterior fossa ependymoma, group PFA
    - Posterior fossa ependymoma, group PFB
    - Spinal ependymoma
    - Spinal ependymoma, *MYCN*-amplified
    - Myxopapillary ependymoma
    - Subependymoma
- Choroid plexus tumors
  - Choroid plexus papilloma
  - Atypical choroid plexus papilloma
  - Choroid plexus carcinoma
- Embryonal tumors
  - Medulloblastoma
    - Medulloblastomas, molecularly defined
      - Medulloblastoma, WNT-activated
      - Medulloblastoma, SHH-activated and *TP53*-wildtype
      - Medulloblastoma, SHH-activated and *TP53*-mutant
      - Medulloblastoma, non-WNT/non-SHH
    - Medulloblastomas, histologically defined
  - Other CNS embryonal tumors
    - Atypical teratoid/rhabdoid tumor
    - *Cribriform neuroepithelial tumor*
    - Embryonal tumor with multilayered rosettes
    - CNS neuroblastoma, *FOXR2*-activated
    - CNS tumor with *BCOR* internal tandem dupli
    - CNS embryonal tumor
- Pineal tu
  - myxoid tumor of the pineal region, *SMARCB1*-mutant
- Cranial and paraspinal nerve tumors
  - Schwannoma
  - Neurofibroma
  - Perineurioma
  - Hybrid nerve sheath tumor
  - Malignant melanotic nerve sheath tumor
  - Malignant peripheral nerve sheath tumor
  - Paraganglioma
- Meningiomas
  - Meningioma
- Mesenchymal, non-meningothelial tumors
  - Soft tissue tumors
    - Fibroblastic and myofibroblastic tumors
      - Solitary fibrous tumor
    - Vascular tumors
      - Hemangiomas and vascular malformations
      - Hemangioblastoma
    - Skeletal muscle tumors
      - Rhabdomyosarcoma
    - Uncertain differentiation
      - *Intracranial mesenchymal tumor, FET-CREB fusion-positive*
      - *CIC*-rearranged sarcoma
      - Primary intracranial sarcoma, *DICER1*-mutant
      - Ewing sarcoma
- Chondro-osseous tumors
  - Chondrogenic tumors
    - Mesenchymal chondrosarcoma
    - Chondrosarcoma
  - Notochordal tumors
    - Chordoma (including poorly differentiated chordoma)
- Melanocytic tumors
  - Diffuse meningeal melanocytic neoplasms
    - Meningeal melanocytosis and meningeal melanomatosis
  - Circumscribed meningeal melanocytic neoplasms
    - Meningeal melanocytoma and meningeal melanoma
- Hematolymphoid tumors
  - Lymphomas
    - CNS lymphom
      - na of the CNS
      - ymphoma
      - phomas in the CNS
      - ALT lymphoma of the dura
      - Other low-grade B-cell lymphomas of the CNS
      - Anaplastic large cell lymphoma (*ALK+/ALK–*)
      - T-cell and NK/T-cell lymphomas
  - Histiocytic tumors
    - Erdheim-Chester disease
    - Rosai-Dorfman disease
    - Juvenile xanthogranuloma
    - Langerhans cell histiocytosis
    - Histiocytic sarcoma
- Germ cell tumors
  - Mature teratoma
  - Immature teratoma
  - Teratoma with somatic-type malignancy
  - Germinoma
  - Embryonal carcinoma
  - Yolk sac tumor
  - Choriocarcinoma
  - Mixed germ cell tumor
- Tumors of the sellar region
  - Adamantinomatous craniopharyngioma
  - Papillary craniopharyngioma
  - Pituicytoma, granular cell tumor of the sellar region, and spindle cell oncocytoma
  - Pituitary adenoma/PitNET
  - Pituitary blastoma
- Metastases to the CNS
  - Metastases to the brain and spinal cord parenchyma
  - Metastases to the meninges

MANY SUBTYPES

Louis *et al.* Neuro Oncol 2021;23(8):1231-1251

Classifier for CNS tumors



Uses 10 000 CpG sites (←→ 850 000 CpG sites on the array)

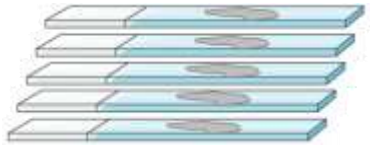
Classifier:

- Developed by using a reference set of 2 801 brain tumors

- Consists of several classes:
  - 182 classes of brain tumors
  - 10 different control tissues

Capper *et al.* Nature 2018;555(7697):469-474.
Capper *et al.* Acta Neuropathol 2018;136(2):181-210.

Methylation array

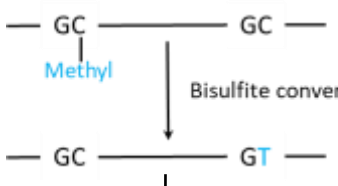


FFPE tissue
(>70% TC)

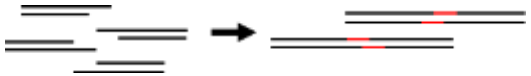
DNA extraction

Tumoral DNA (500 ng)

Bisulfite conversion



Restoration



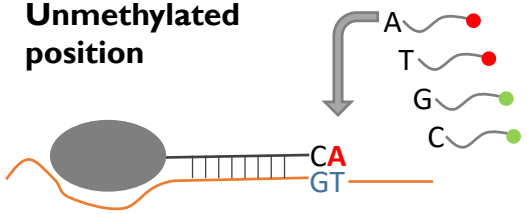
Restored DNA

Methylation array

Amplification and fragmentation

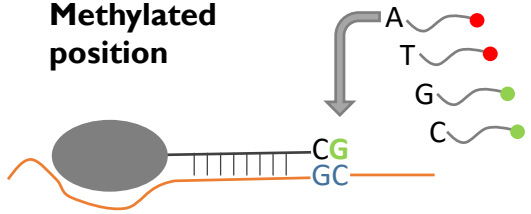
Hybridisation on Infinium EPIC 850k array

Unmethylated position



Bisulfite-treated DNA

Methylated position



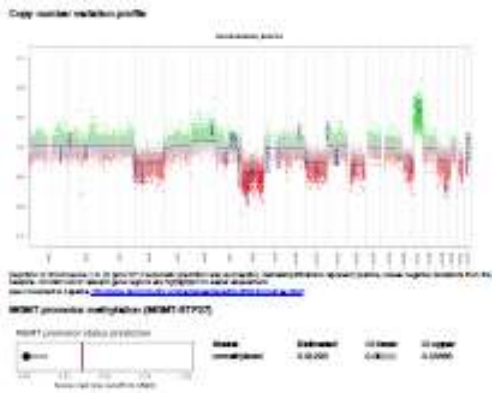
Bisulfite-treated DNA

Read array with iScan machine

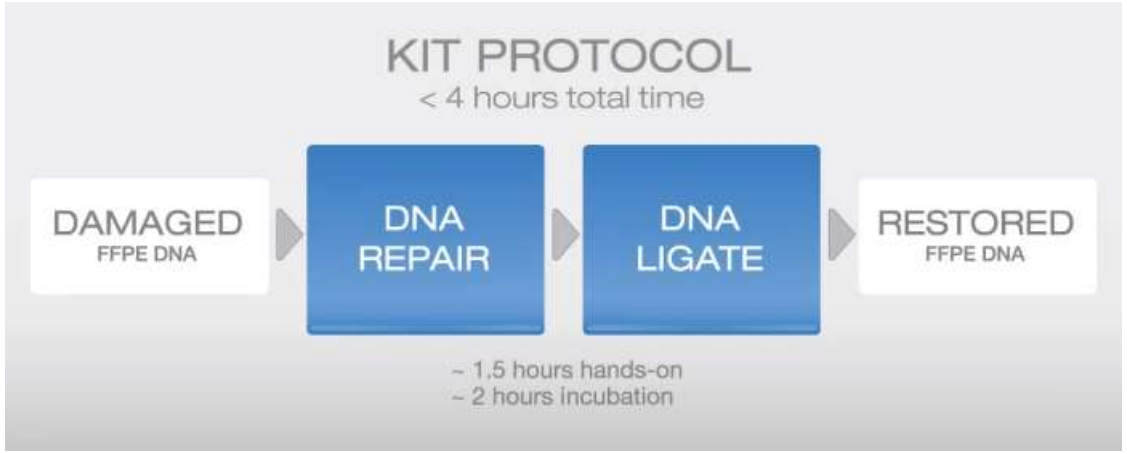
Sample_red.idat + Sample_grn.idat

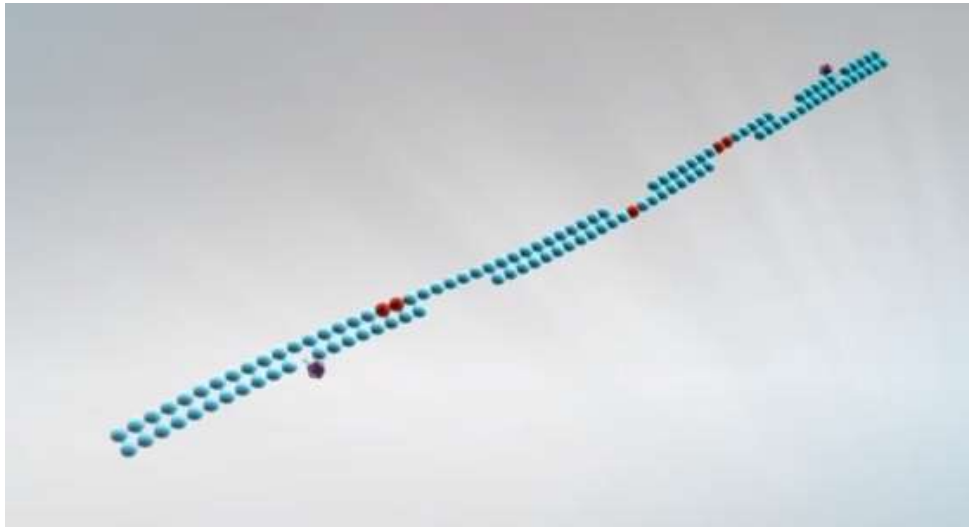
Molecularneuropathology.org

Report:
- Tumor classification based on methylation
- Copy number profile
- MGMT promoter methylation



Methylation array: Restore protocol





DNA from FFPE tissue can be heavily damaged:

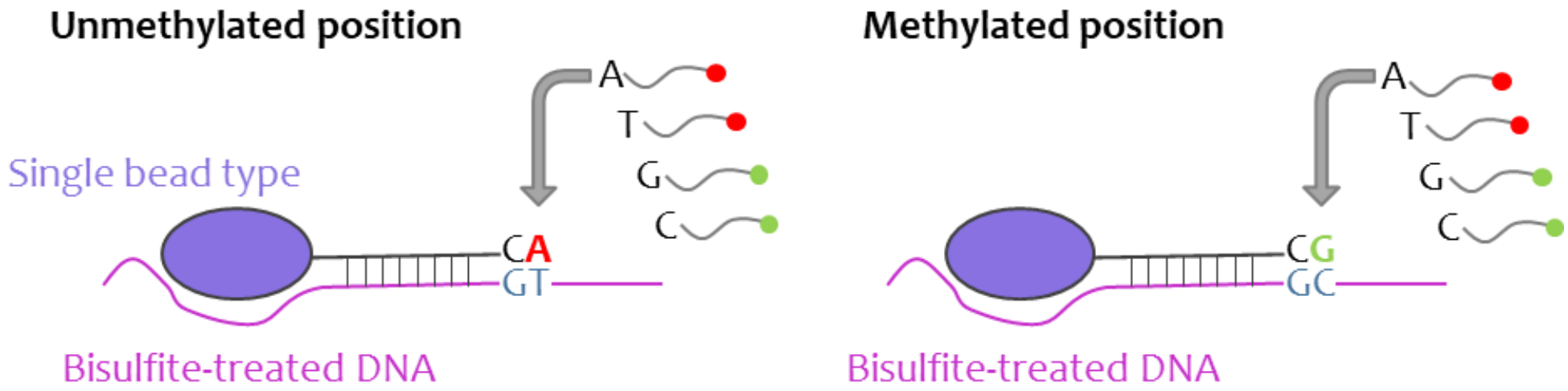
- DNA fragmentation
- Base lesions
- Modified bases
- Cross-linking

https://www.youtube.com/watch?v=V8KvG3aXtOY

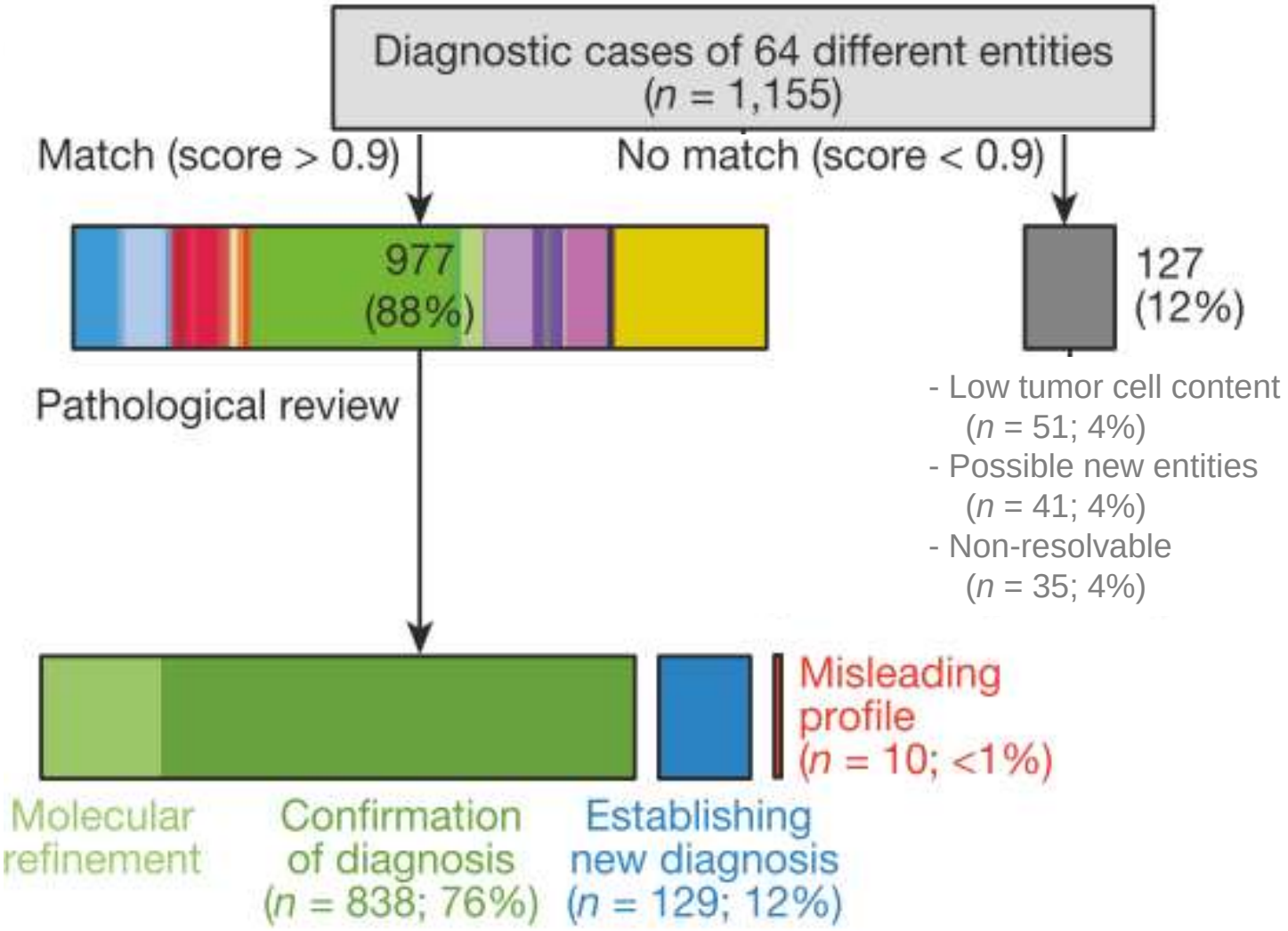
Methylation array: probes

3’ end of each probe complements the base directly upstream of the query site. A single base extension results in the addition of

- a labeled A, complementary to the unmethylated T
- a labeled G, complementary to the methylated C

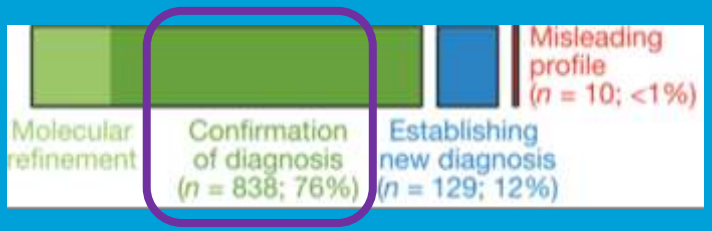


Methylation array: output of the brain classifier



Capper *et al*. Nature 2018;555(7697):469-474.

Methylation array: examples



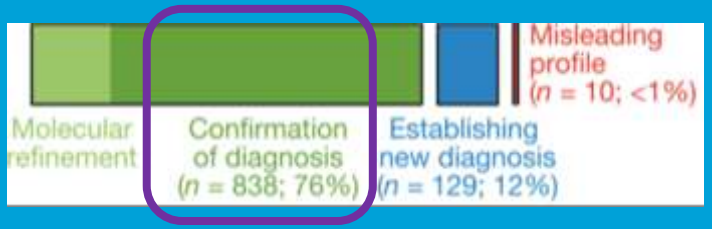
	Male, 60 years old
APO diagnosis	Glioblastoma, WHO grade 4
Molecular tests	FISH EGFR: strong EGFR amplification in 16% of cells IHC IDH1: wildtype

Brain tumor methylation classifier results (v11b4)

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, subclass mesenchymal	0.96	match	●

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)

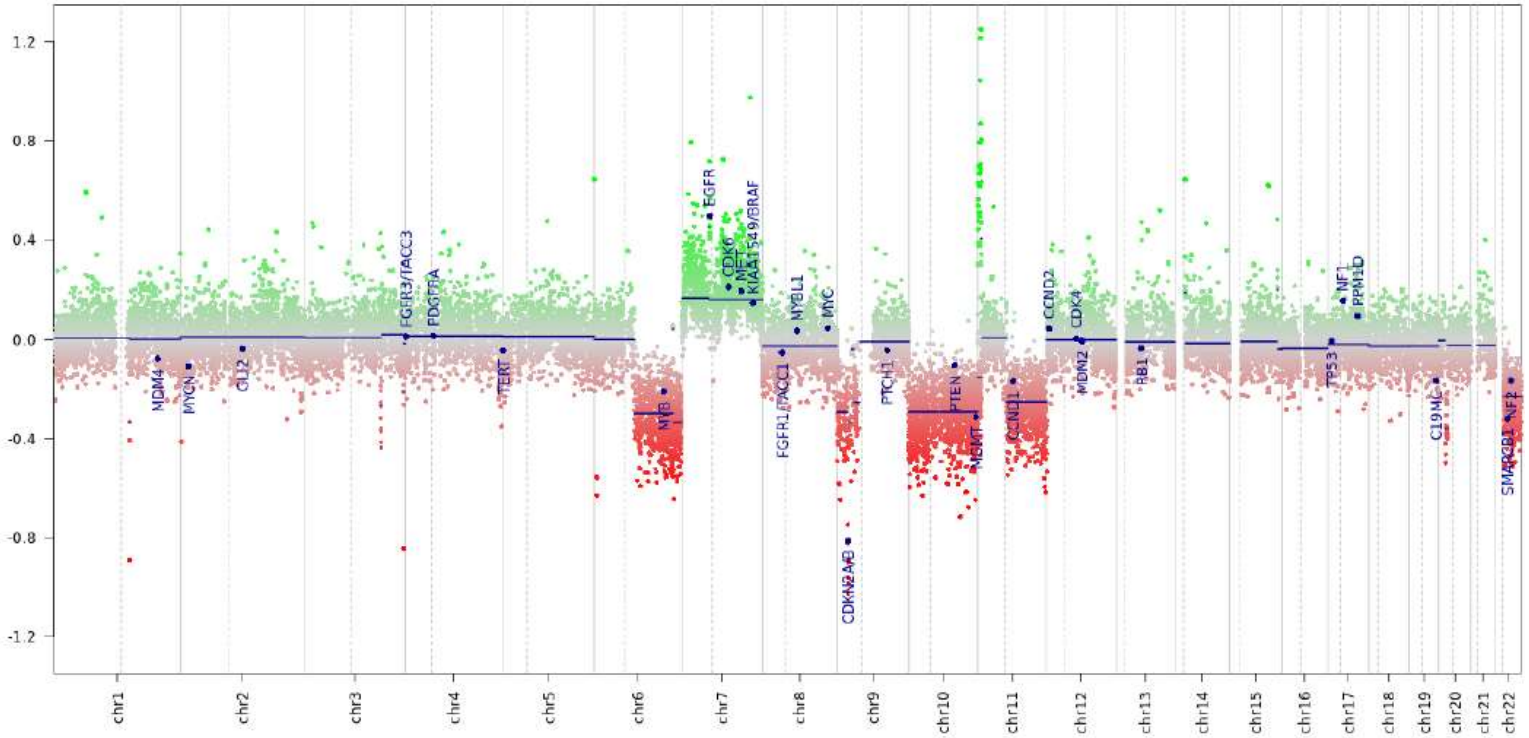
Methylation array: examples



Class descriptions

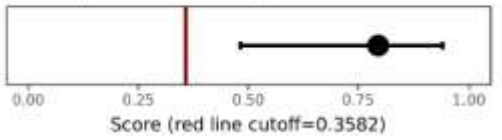
Methylation class family Glioblastoma, IDH wildtype: The methylation class family "Glioblastoma, IDH wildtype" comprises the methylation classes glioblastoma, IDH wildtype, subtype RTK I to III, glioblastoma, IDH wildtype, subtype mesenchymal, glioblastoma, IDH wildtype, subtype MYCN and glioblastoma,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

Methylation class glioblastoma, IDH wildtype, subclass mesenchymal: The methylation class "glioblastoma, IDH wildtype, subclass mesenchymal" is comprised of tumors with a histological diagnosis of glioblastoma or occasionally gliosarcoma. These tumors are typically located in the cerebral hemispheres. Median age is 59 years (range 40 to 86). Recurrent chromosomal alterations are gain of chromosome 7 with or without EGFR amplification (>80%), loss of 9p21 (CDKN2A/B; >60%) and chromosome 10 loss (>90%). Alterations of NF1 may also be enriched in this subtype, and expression profiles often resemble the 'Mesenchymal' subgroup according to the TCGA classification.



MGMT promotor methylation (MGMT-STP27)

MGMT promotor status prediction



Status	Estimated
methylated	0.79575

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

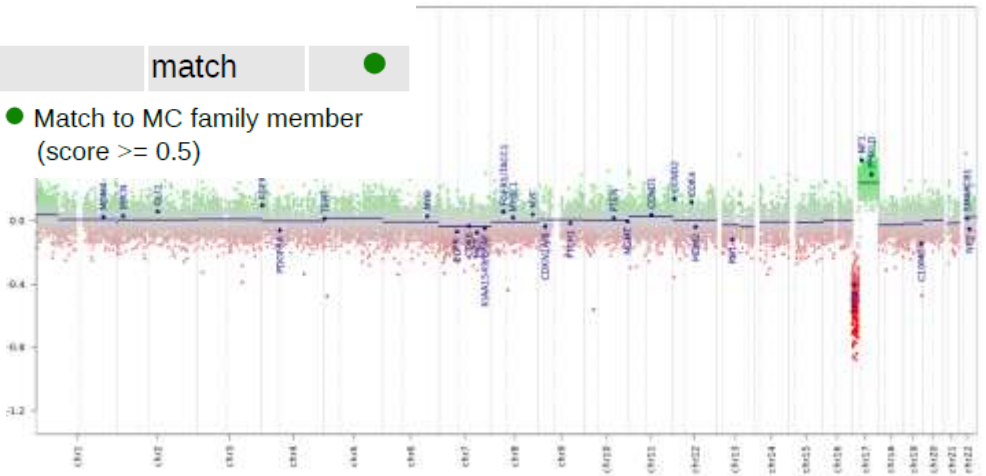
Methylation array: examples

	Girl, 11 years old
APO diagnosis	Medulloblastoma, WHO grade 4, group 3 or 4?
Molecular tests	FISH: no amplification of NMYC of MYC FISH: i(17q) in 54% of cell nuclei DNA sequencing: no mutations in CTNNB1, PTCH1, SMO, SUFU or TP53

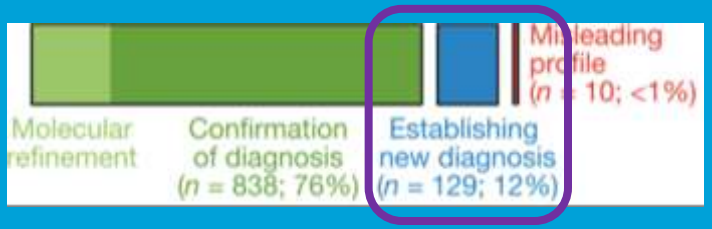
Brain tumor methylation classifier results (v11b4)

Methylation classes (MCs with score >= 0.3)	Calibrated score	Interpretation	
methylation class family Medulloblastoma group 3 and 4	0.99	match	✔
MC family members with score >= 0.1			
methylation class medulloblastoma, subclass group 4	0.99	match	●

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)



Methylation array: examples



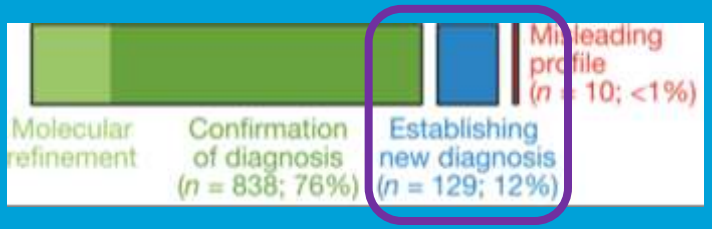
	Male, 58 years old
APO diagnosis	Diffuse type glioma, preference astrocytoma, at least WHO grade 3
Molecular tests	FISH: no 1p/19q co-deletion DNA sequencing: no IDH1/2 mutation

Brain tumor methylation classifier results (v11b4)

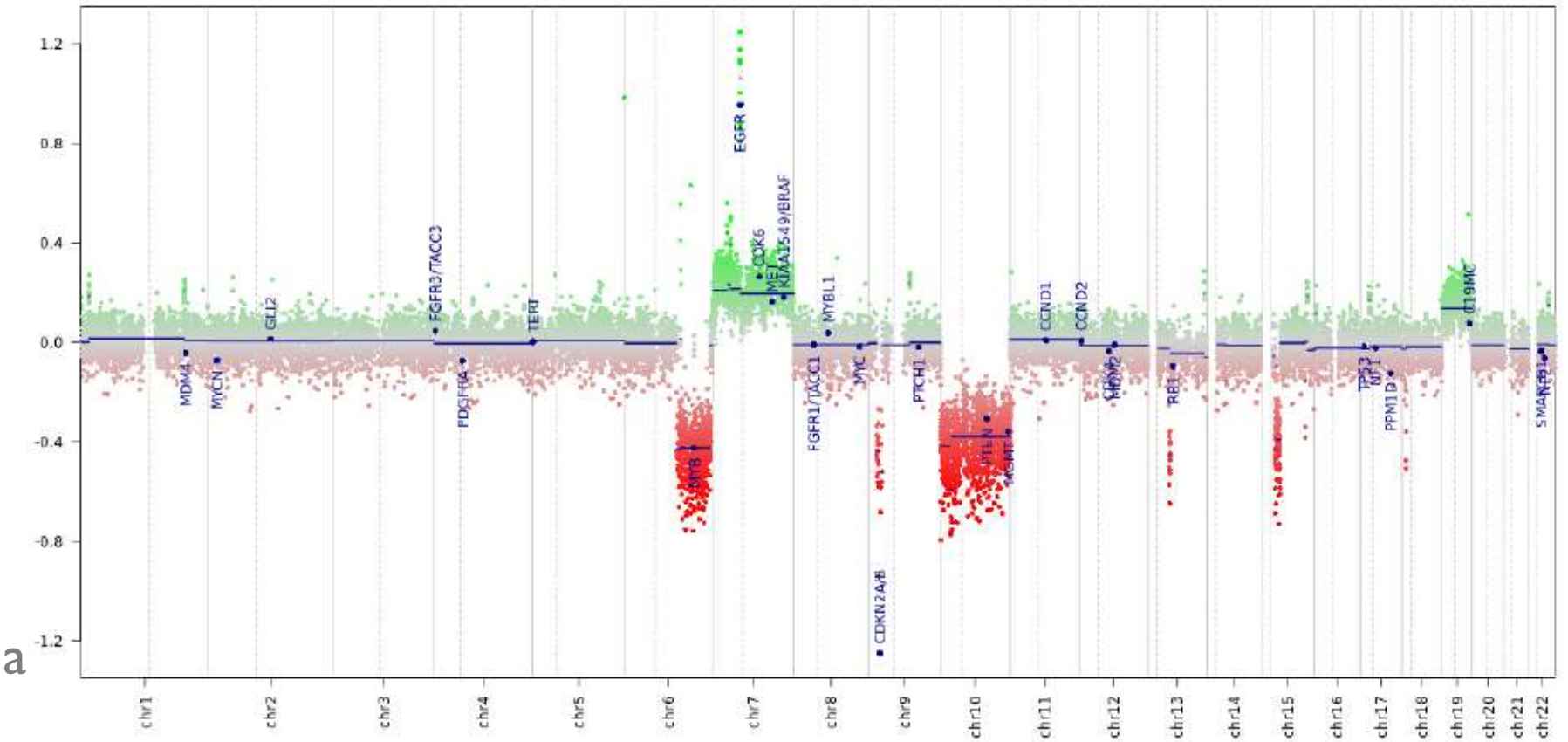
Methylation classes (MCs with score >= 0.3)	Calibrated score	Interpretation	
methylation class family Glioblastoma, IDH wildtype	0.99	match	✔
MC family members with score >= 0.1			
methylation class glioblastoma, IDH wildtype, subclass RTK II	0.72	match	●
methylation class glioblastoma, IDH wildtype, subclass 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)

Methylation array: examples



If presence of
- EGFR amplification
- And gain of chr 7
- And loss of chr 10



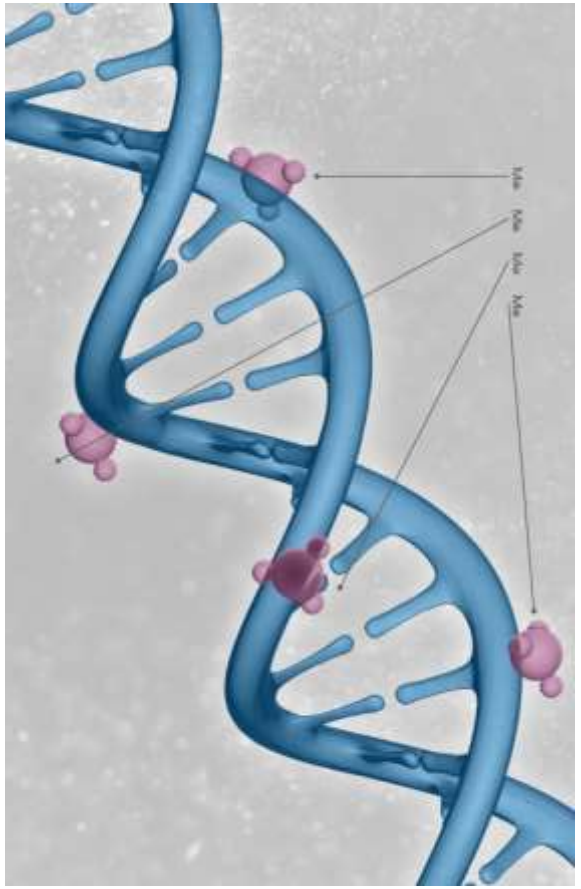
→ re-classification from anaplastic astrocytoma to glioblastoma

→ Classifier is correct

Stichel *et al.* Acta Neuropathol. 2018;136(5):793-803.

Conclusions

- Epigenetic changes
  - Have key pathophysiological roles in the initiation and progression of cancer
  - Are biomarkers for diagnosis, prognosis and prediction of treatment response
  - Are reversible and attractive targets for further cancer treatments



"Epigenetica zijn alle eigenaardige en wonderbaarlijke dingen die niet door genetica verklaard kunnen worden."

Denise Barlow