



Garvan Institute
of Medical Research



Epigenetics, Epigenomics and its applications

March 01 2025

Phuc Loi Luu, PhD

Email: luu.p.loi@googlemail.com

0901802182

Acknowledgement



All patients who donate samples

All member of Prof. Susan Clark lab

- Prof. Susan Clark
- Dr Clare
- Dr Ruth
- Jenny
- Wenija
- Dilys
- Dr Qian
- Dr Amanda
- Dr Jo
- Dr Braydon
- Dr Shalima



Acknowledgement collaborators and students in Viet Nam

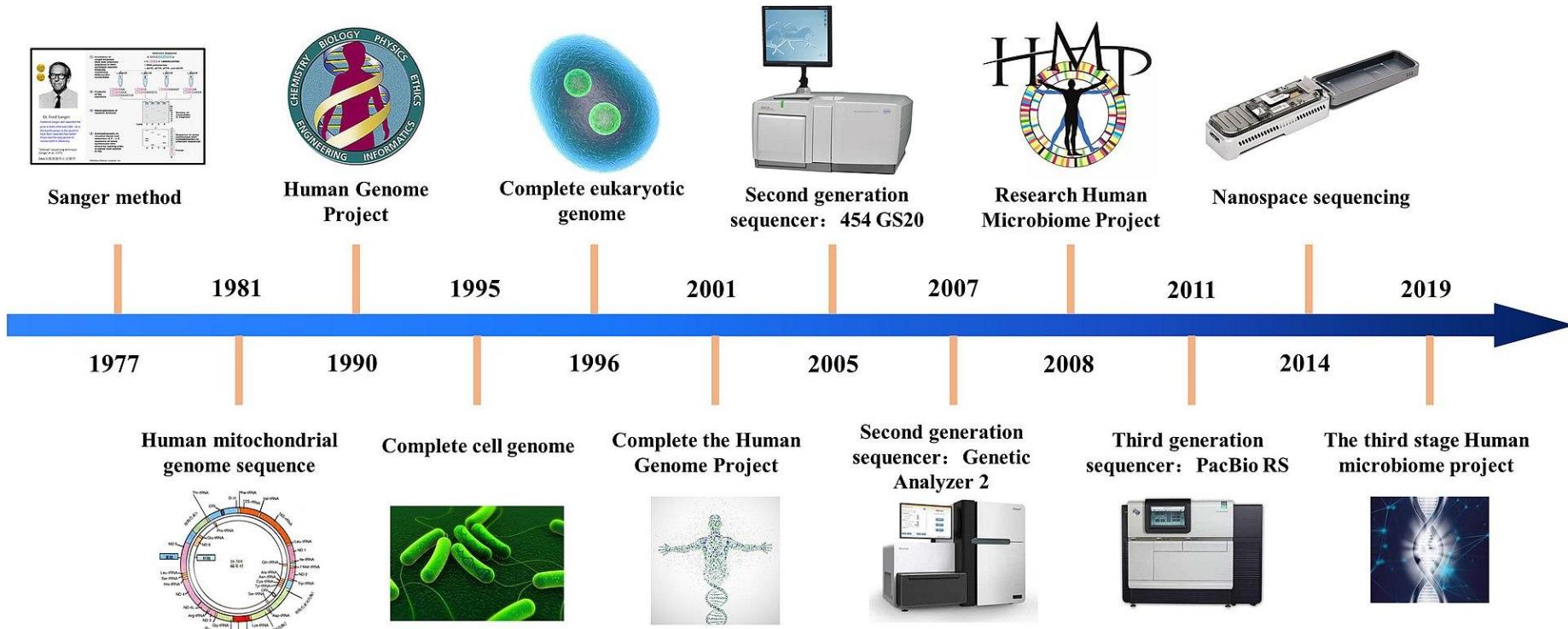
- NGUYỄN LÊ ĐỨC MINH, MD
- ĐÀO DUY KHƯƠNG
- NGÔ ĐẠI PHÚ
- ÔNG HỒNG
- HOÀNG KIM
- NGUYỄN VĂN GIÀU
- HÀ HUY
- NGUYỄN MẠNH HÙNG
- TRẦN THỊ THANH KHƯƠNG, PhD
- CAO THỊ TÀI NGUYÊN, PhD
- TRỊNH VẠN NGŨ, PhD
- Bsc. LÊ NHẤT THÔNG
- Bsc. PHẠM MAI TÂM
- Bsc. NGUYỄN TẤN THANH GIANG
- Bsc. NGUYỄN ANH XUÂN
- Bsc. NGUYỄN MINH HOÀNG
- Bsc. TRẦN BÁ THIỀN
- ÔNG PHÚC THỊNH, MD
- NGUYỄN HUY THỊNH, MD
- ĐÀO NGỌC BẮC, MD, Msc
- TRẦN NGUYỄN TRỌNG PHÚ, MD
- Msc. TRẦN MINH
- Tuệ An

All member of VnPathoinformatics group

Outline

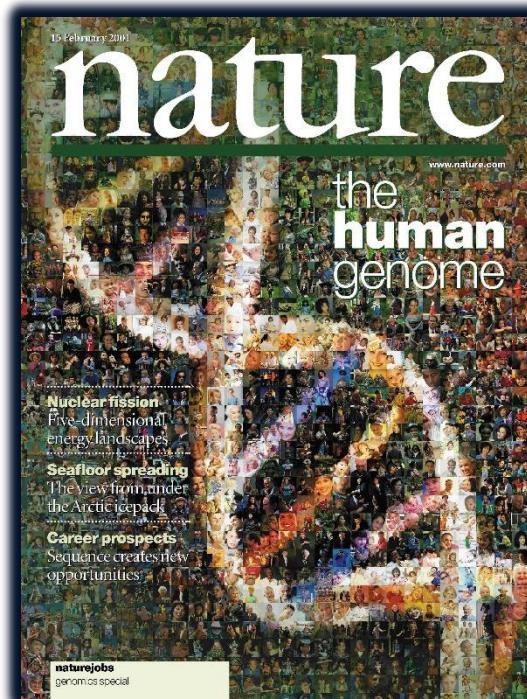
- What is Epigenetics, Epigenome and Epigenomics?
- How does Epigenome regulate transcription?
- Clinical translation I: pharmaco-epigenomics with DAC in breast cancer
- Clinical translation II:
 - mGASTP1 headloop PCR assay for monitoring for treatment metastatic Castration-resistant prostate cancer with docetaxel
 - Liquid biopsy (cfDNA) in breast cancer diagnosis
 - Prognostic epigenetic biomarkers for prostate cancer mortality
 - COVID19 DNA methylation risk (COMER) score for severity classification

High-throughput sequencing (HTS) methods

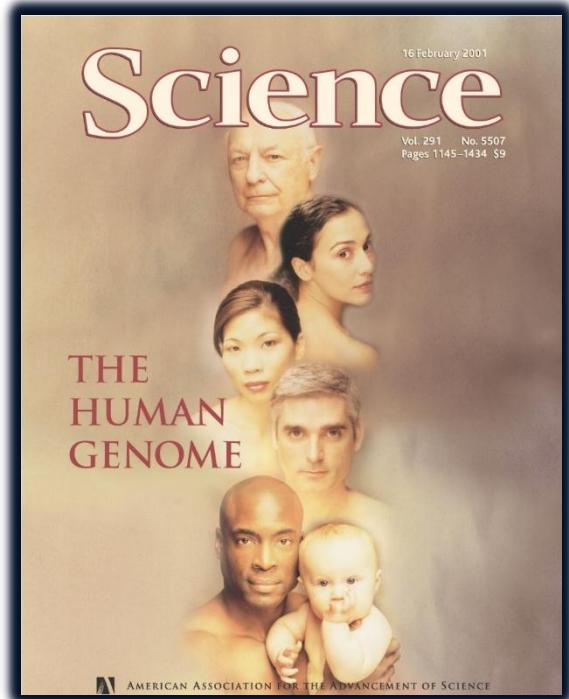


Dự án hệ gen người HGP (Oct 1990 - April 2003)

- Năm 2003, dự án hệ gen người HGP đã tạo ra một chuỗi trình tự gần 3 tỉ nucleotide chiếm hơn 90% bộ gen người.
- Đây là chuỗi gen hoàn chỉnh nhất có thể đạt được với công nghệ giải trình tự DNA thời điểm đầu những năm 2000.
=> Sự phát triển của công nghệ giải trình tự thế hệ mới (NGS).



HGP Paper



Venter/Celera Paper

Human Genome Build

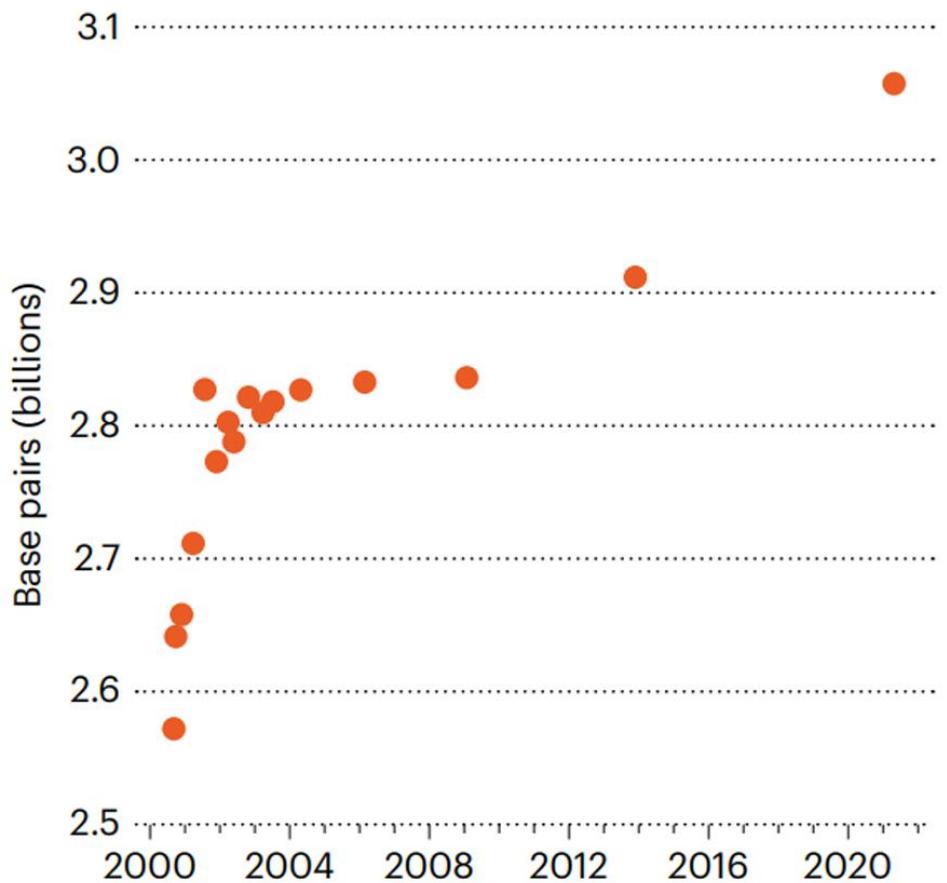
Human	hs1	Jan. 2022	T2T Consortium CHM13v2.0	Available
	hg38	Dec. 2013	Genome Reference Consortium GRCh38	Available
	hg19	Feb. 2009	Genome Reference Consortium GRCh37	Available
	hg18	Mar. 2006	NCBI Build 36.1	Available
	hg17	May 2004	NCBI Build 35	Available
	hg16	Jul. 2003	NCBI Build 34	Available
	hg15	Apr. 2003	NCBI Build 33	Archived
	hg13	Nov. 2002	NCBI Build 31	Archived
	hg12	Jun. 2002	NCBI Build 30	Archived
	hg11	Apr. 2002	NCBI Build 29	Archived (data only)
	hg10	Dec. 2001	NCBI Build 28	Archived (data only)
	hg8	Aug. 2001	UCSC-assembled	Archived (data only)
	hg7	Apr. 2001	UCSC-assembled	Archived (data only)
	hg6	Dec. 2000	UCSC-assembled	Archived (data only)
	hg5	Oct. 2000	UCSC-assembled	Archived (data only)
	hg4	Sep. 2000	UCSC-assembled	Archived (data only)
	hg3	Jul. 2000	UCSC-assembled	Archived (data only)
	hg2	Jun. 2000	UCSC-assembled	Archived (data only)
	hg1	May 2000	UCSC-assembled	Archived (data only)

A COMPLETE HUMAN GENOME IS CLOSE: HOW THE GAPS WERE FILLED

Researchers added 200 million DNA base pairs and 115 genes – but they've yet to finish the Y chromosome.

COMPLETING THE HUMAN GENOME

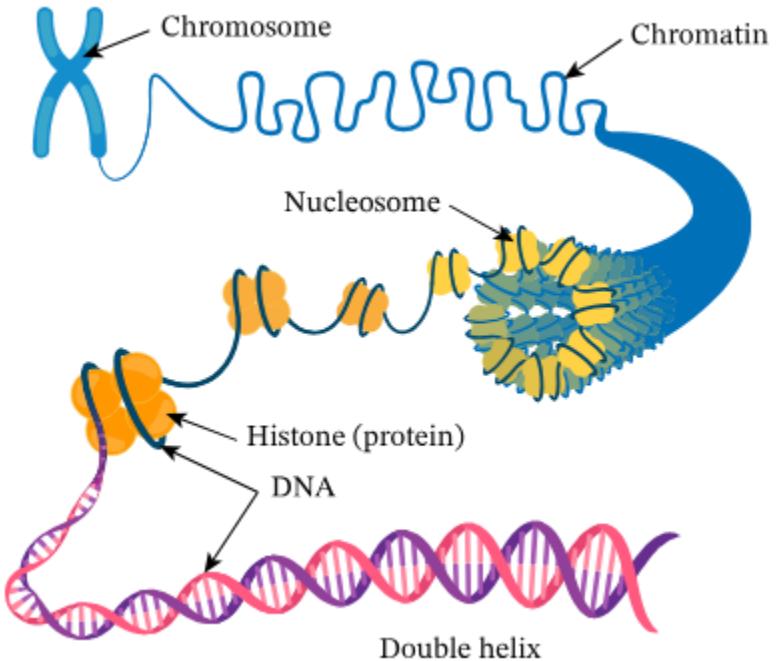
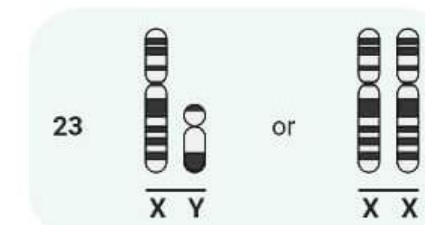
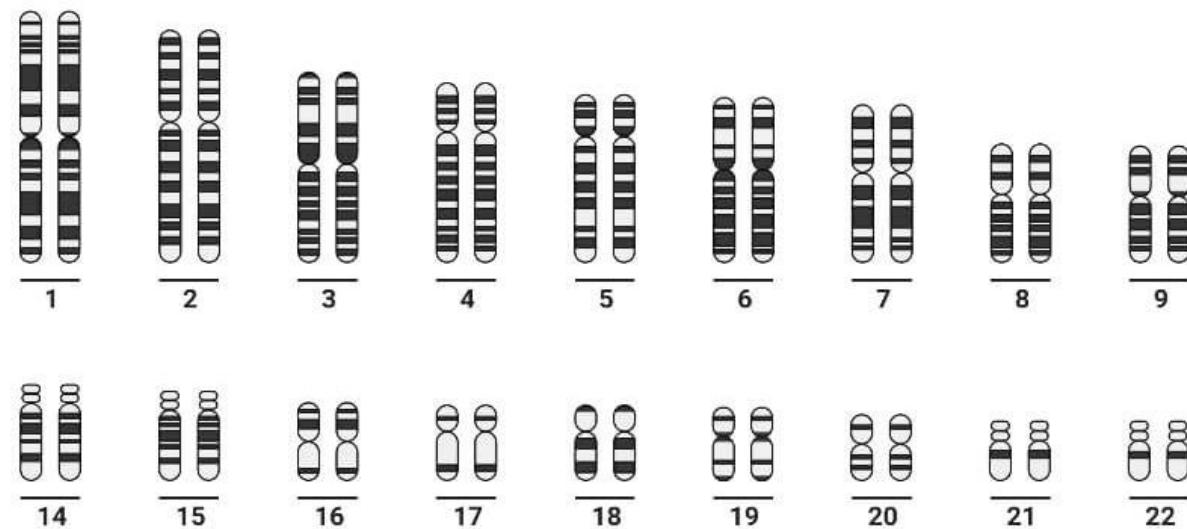
Researchers have been filling in incompletely sequenced parts of the human reference genome for 20 years, and have now almost finished it, with 3.05 billion DNA base pairs.



0.3% of sequence might still have errors. Includes X but not Y chromosome. Count excludes mitochondrial DNA.

SOURCE: ADAM PHILLIPY

Human Karyotype





PACIFIC INFORMATICS
Your Trusted Partner in Bioinformatics

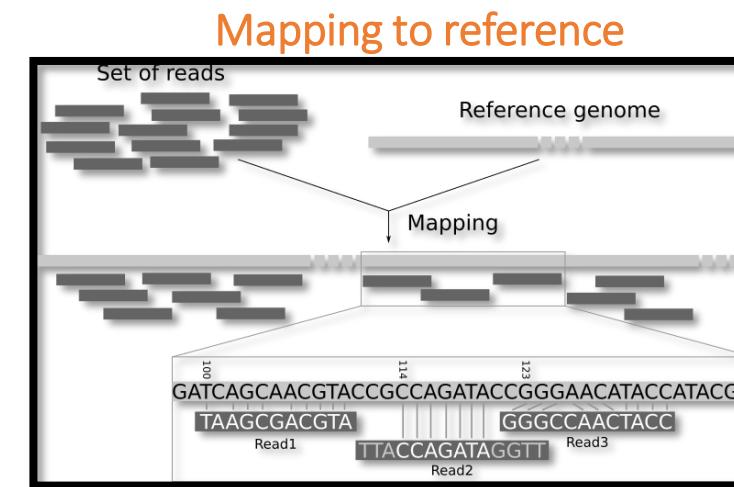
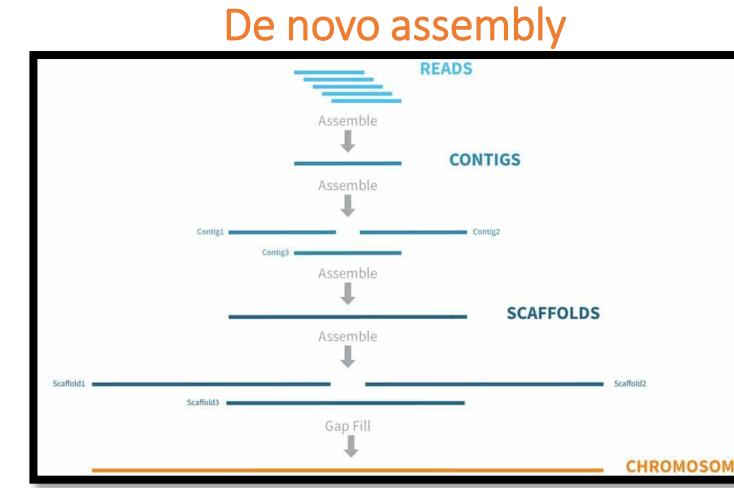
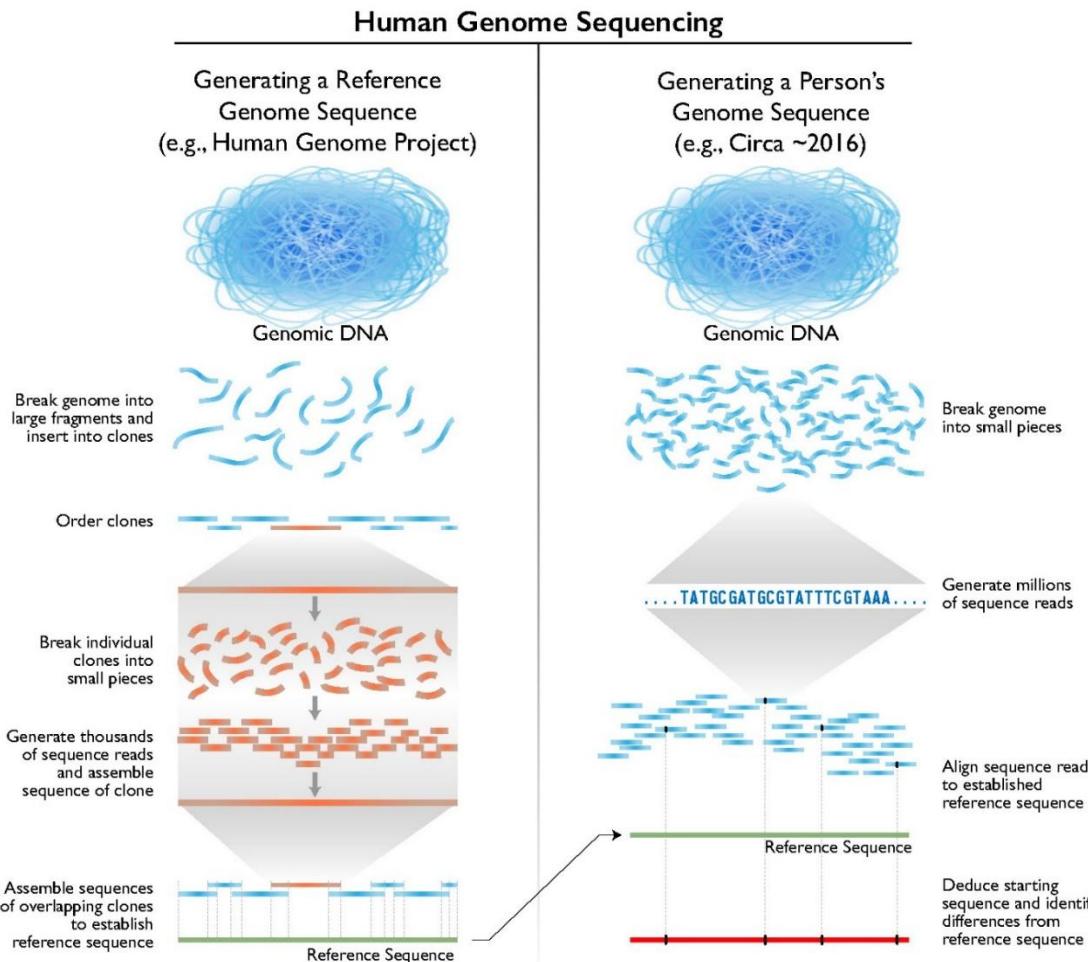


48541 agcccttcaa agaaatgttc tcagcaggca tggagcccag gacttgctcc ctttgggtag
48601 agagccgggt tgaaggtgac tgaagtgaaa tgggacagta gaggcgaaaa gggtgttag
48661 ttccctggagg tgggggtgt gggAACCTGC tttgtactga gatgcacccc tgccagttct
48721 gcctgaagat ttgaggcggg gggcaggggg gcggagtgaa gtcattttac tggtaagtaa
48781 ttttaaacct ttaatatta aagcaaacgt ggatatgtaa tgaatgaaat tcattctgga
48841 atgaaaaatt cacgtatgt taaaaataaa cacggggctt cagagaggac tttctggctg
48901 gcagcagact ccagattccc agggccctg caccctcctc tgcccacagg gcacctaatt
48961 ggagaaggtg tgggaggaga gccaggccgg agtcagagca cactggtgc tccacatttgc
49021 cagcgtgccc tgcctctc ctgaggctt gcaacgtgca atatgctaag caaactcccc
49081 ctgtccccgt ccagttctg aggacaagag ccaccacctg tagcaaataa agaccaggca
49141 accctttgac tcatcttgc gagtctctgg aatcagaggg tagccacatc gctgagaggt
49201 ggagtgaagc actcgggtga aaaggtacaa ggaagtcaagg gacaggagtg tggggacatc
49261 acctagacaa tgacagagaa gaggggcaca gccgagttag gggaggggg ccggcagtcc
49321 tacatccccct ggcctgaagc acgctccagg gcagaaggaa aaacactgtc tttgggttcc
49381 aagagacctg agttcaaatt ctggctccac cactgaccac ctgtgttaacc ttgaactgct
49441 gctgcctgaa cctcagggtt cccttctaaa aatagaggag aaaaggatgc atttctcctt
49501 gcccctgtga gaacgaaatg gtgcaagcac caaggagct cagcaaagggt cggcctgcc
49561 cccgcctggc caaaccttc ctcttcaggaa ggccacggca accgtagttt gacagaagag
49621 cagcacctt attaatgtc tccctcaggatc tgtccttgc caagtccacct aacctctctg
49681 ggctgcttcc tcattggaa aatatggctt ccagtaaaac ctggccctgtc cacctcttgg
49741 ggcacttggc aaacagcaaa agagtccaaa tgtcaggctt gggccaggcg cagtggctca
49801 tgcctgtaat cccagcaatt taggaagcca aggtggcggtt atcaccgtt gtcaggagtt
49861 tgagaccagc ctggccaaca tggtaaaacc ttgtctctac aaaaatacaa aaattagccg
49921 gcatgtatgg cgggtgcctg taatccctact tactcggag gctgaggcaaa gagaatcgct
49981 tgaacccggaa agggaaagggt tgcagttagc caagattgtt ccactgcact ccagctgggg
50041 caacagagcg agactctgtc taaaaaaaaaaaaaaa aaaaaaaaaaa aaacaatgca gagctggctg
50101 tgtaaaaaac ctgttccact gcagggccca gtgtccacca ggctgggggtt caggccatag
50161 ggggtgggggc ccagcatcag cctctcaggaa gccctgggg gggggccgc tccctgtcccc
50221 ctcgtggctt ggatgtgttcc tagcccaagt cctagtttac acctgcccgtc gcctggcctc
50281 tcaggagagg cccagggtga ggaggagcat ggttaaagggtt aagctgattt ggaagtccgc
50341 tggggaaa gcaactcctt gcacattggaa ggaacccgaga aagactgacc ccgaggacag
50401 cagccagcat ggccttcctt gggagcccat gttggggat tccctgtgc gccaaggctc
50461 agcccttggc gtcgcagggtt ctggctctgg cctctccccc tcccatgcag gggcaggg
50521 gagatggctt ctgaggacctt gttcagctt tggccctggg aatagattt ccaggagct
50581 ttaaaggcagc tgagtgtgtc atccagctaa gcctggggaa ggagcttggc tcaaggcttgc
50641 acaggtgtga cagggatggg gactggaaag taagagatga aaccctggct ggaggctgtg
50701 agcttccaca gccagcgctt gacaggaggg gtccagatccactatgaccacca

Giới thiệu về

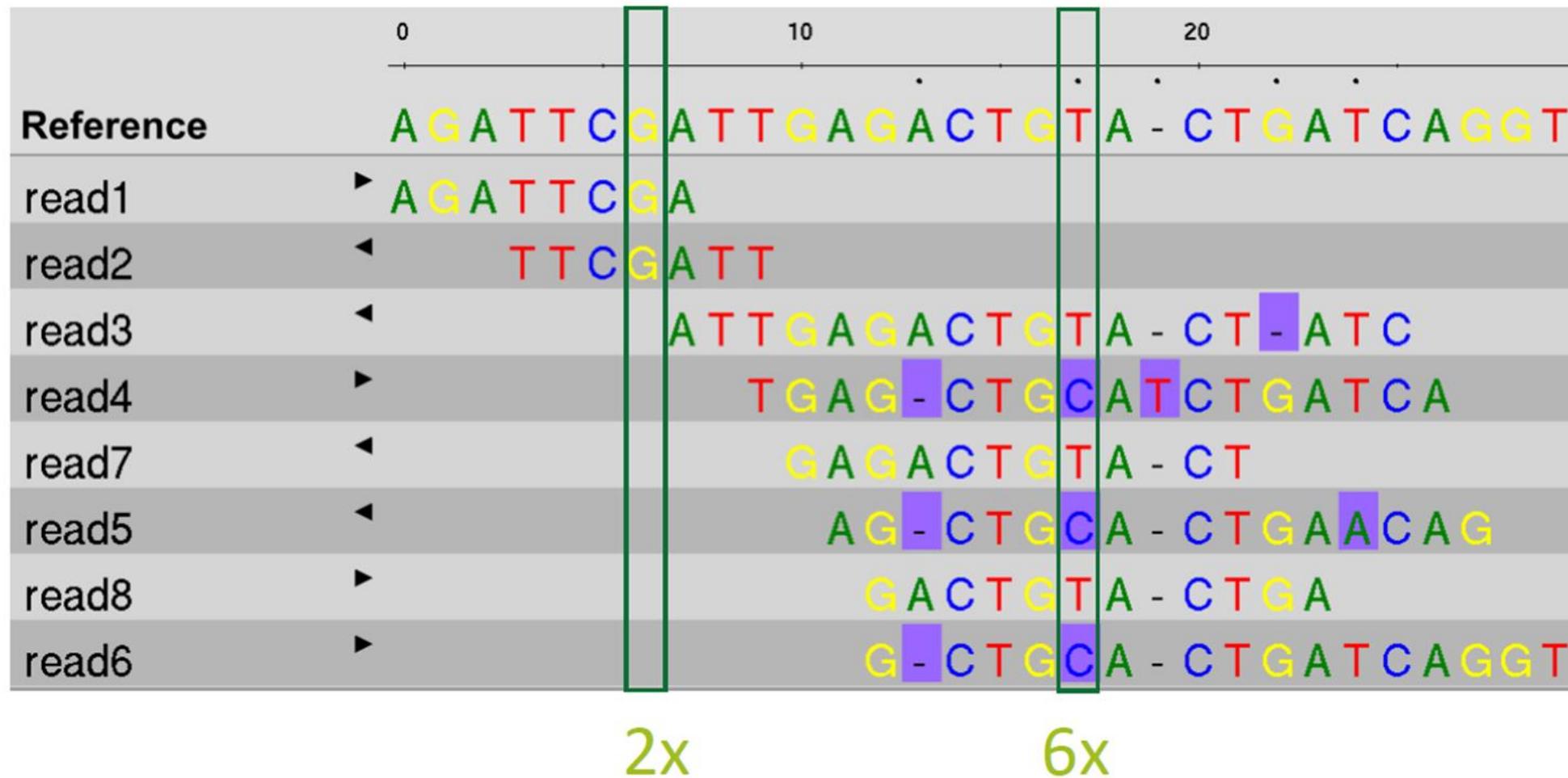
GIẢI TRÌNH TỰ GEN THẾ HỆ MỚI

Giải trình tự gen thế hệ mới (NGS)

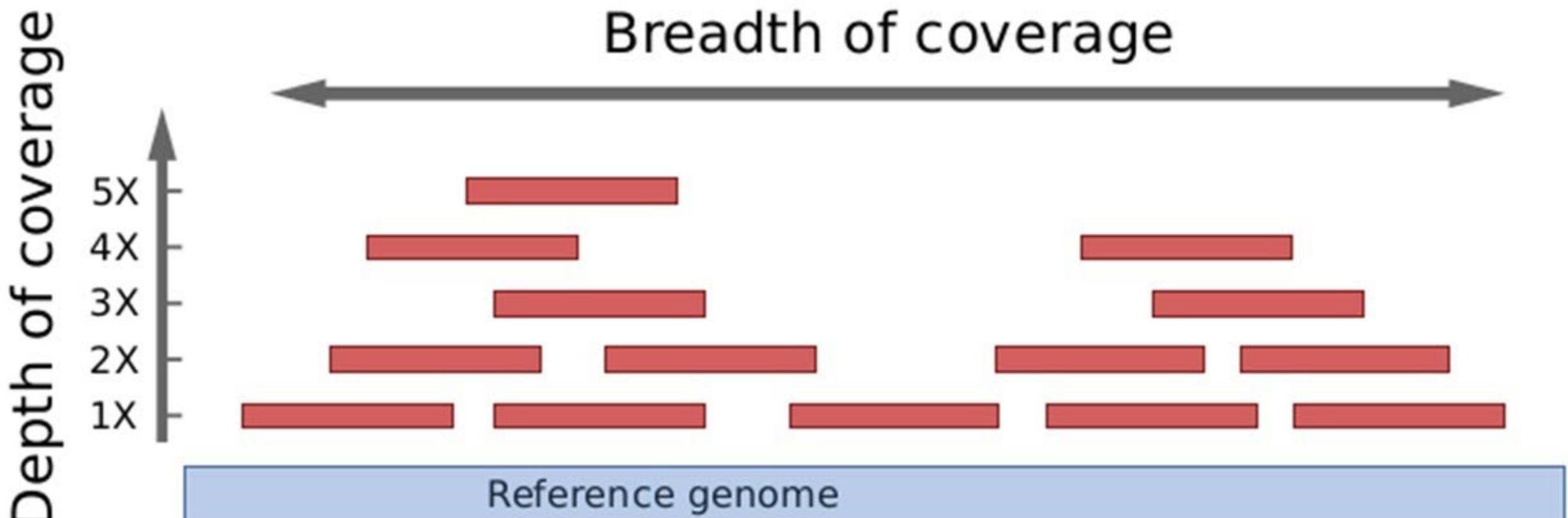


<https://www.genome.gov/about-genomics/fact-sheets/Sequencing-Human-Genome-cost>

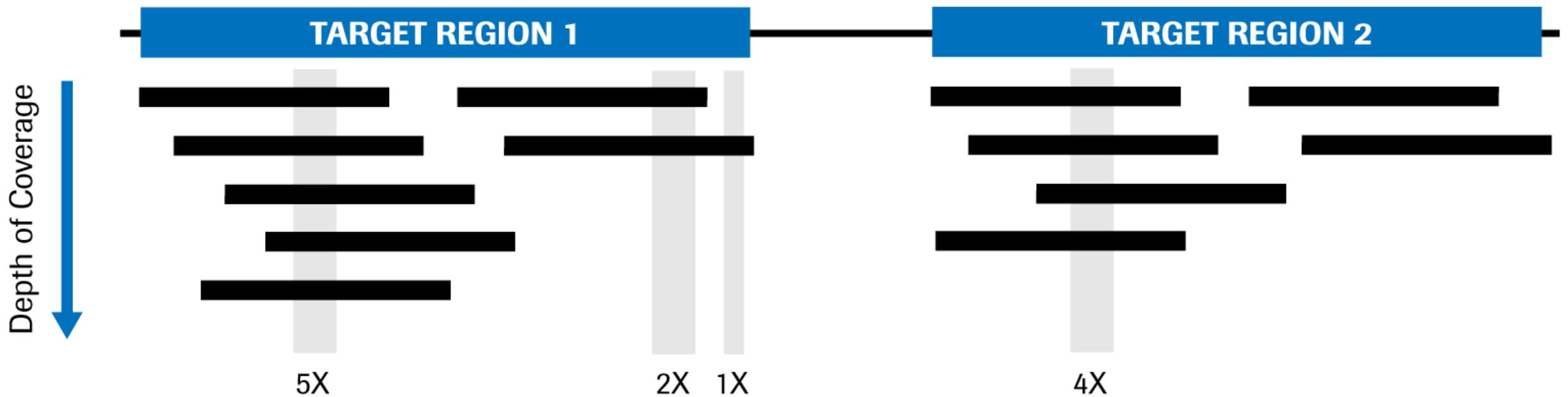
Kết quả của mapping



Depth vs Breadth of Coverage



Depth of Coverage



Giải trình tự gen thế hệ mới (NGS): Có hệ gen tham chiếu

De novo assembly

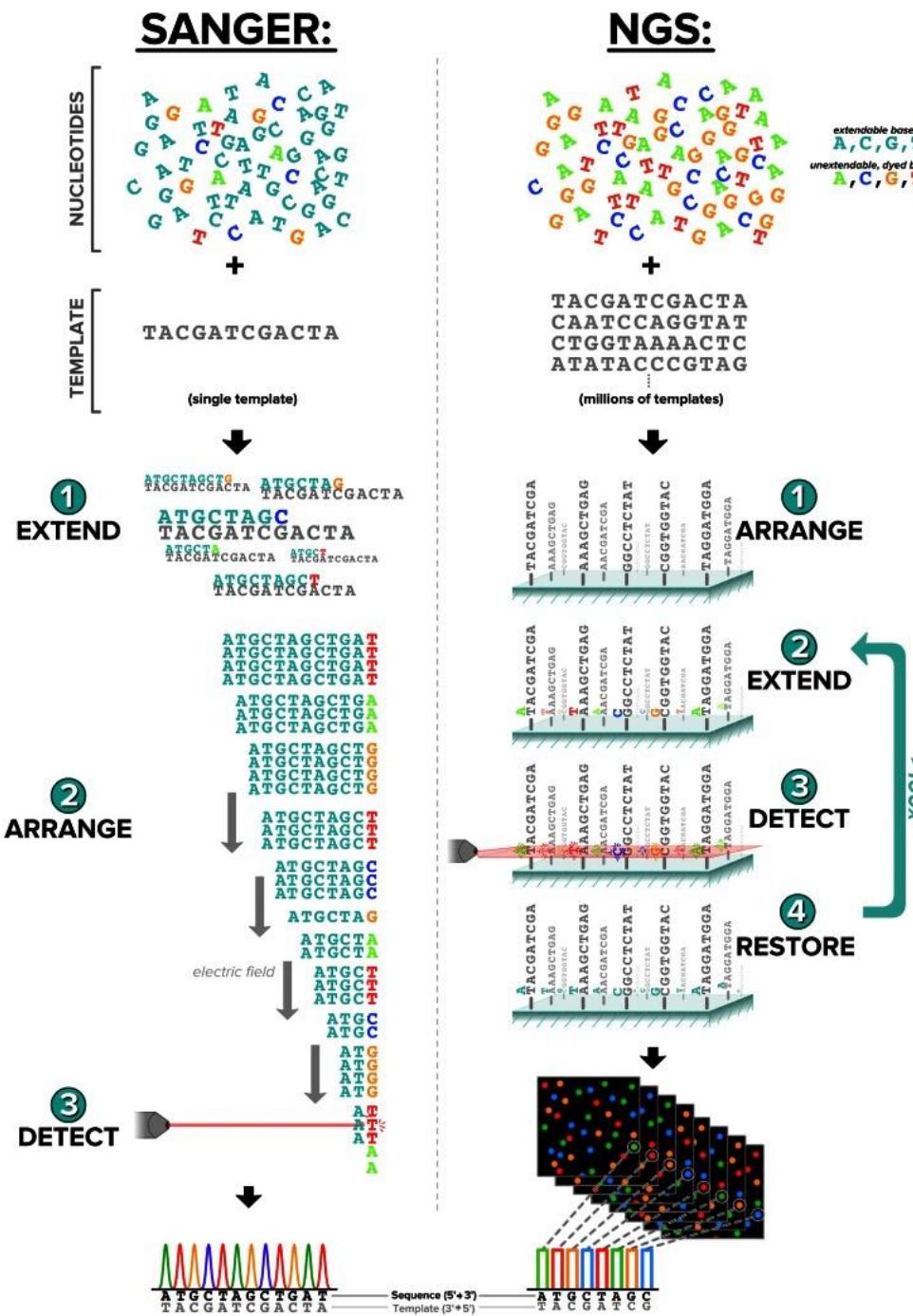


Mapping to reference



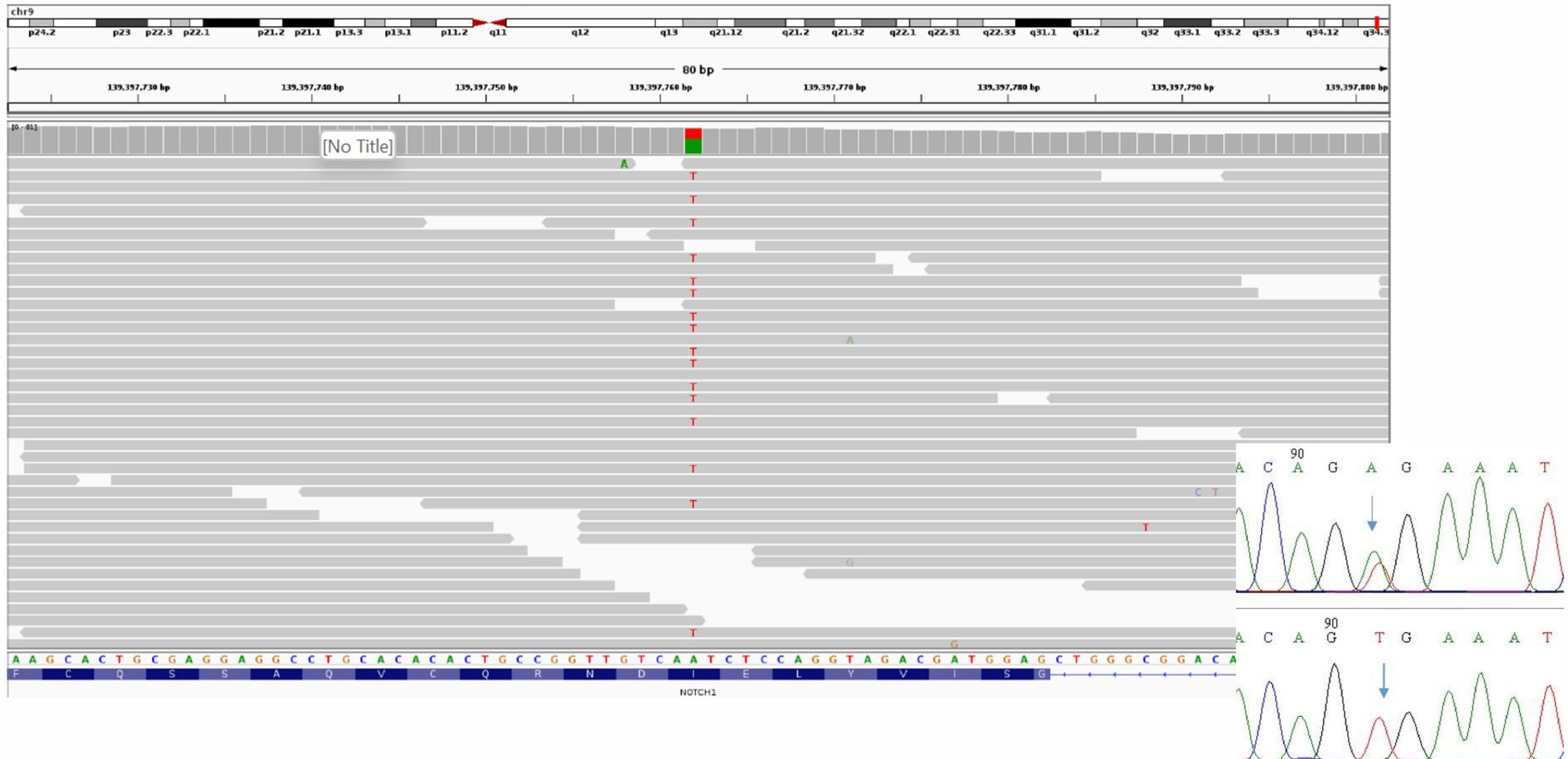
Giải trình tự gen thế hệ mới (NGS): giải trình tự song song

Read1: CTCGAATACG



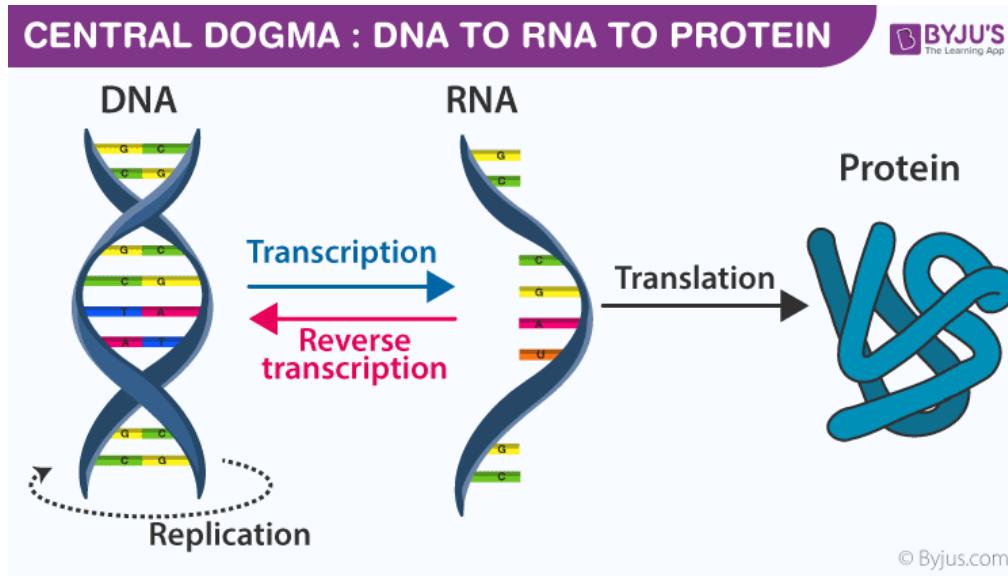
Read1: CTCGAATACG
 Read2: CTCGAATACG
 Read3: CTCGAATACG
 Read4: CTCGAATACG
 Read5: CGCGAATACG
 Read6: CGCGAATACG
 Read7: CGCGACTACG
 Read8: CGCGAATACG

NGS vs Sanger

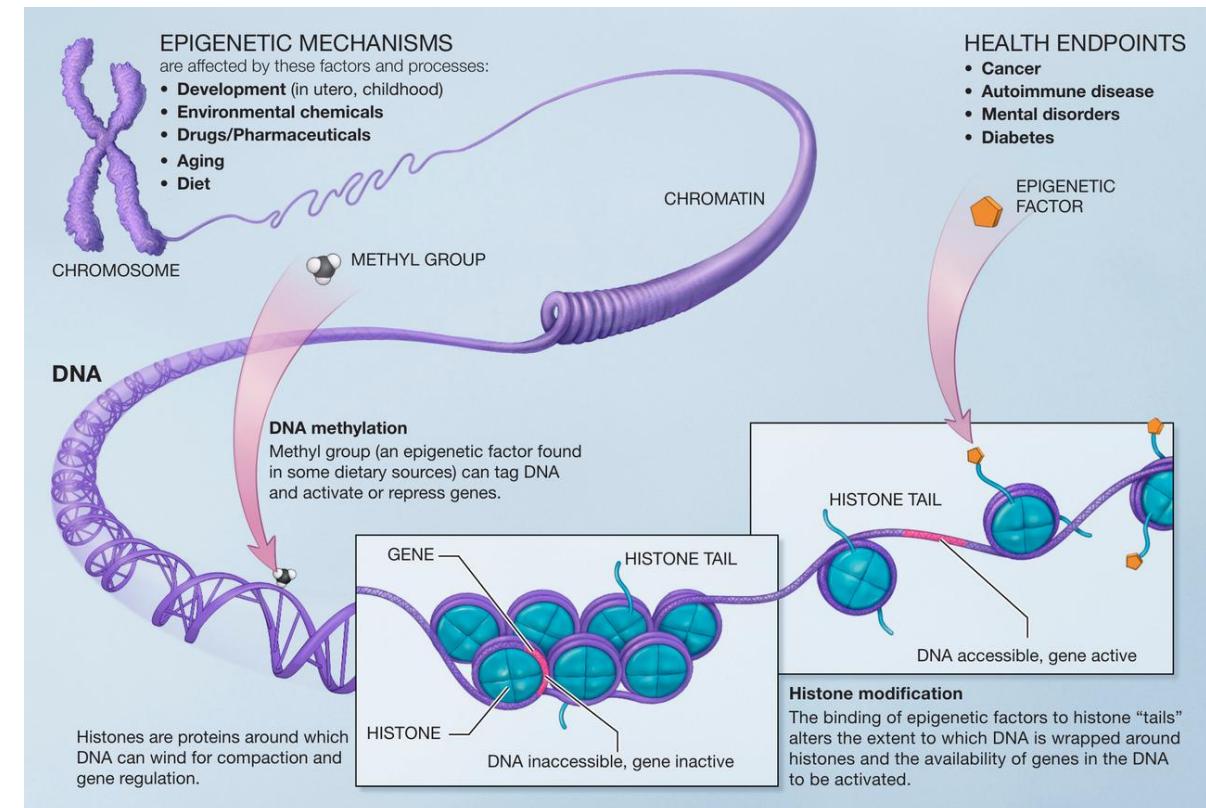


What is Epigenetics?

Your genes play an important role in your health



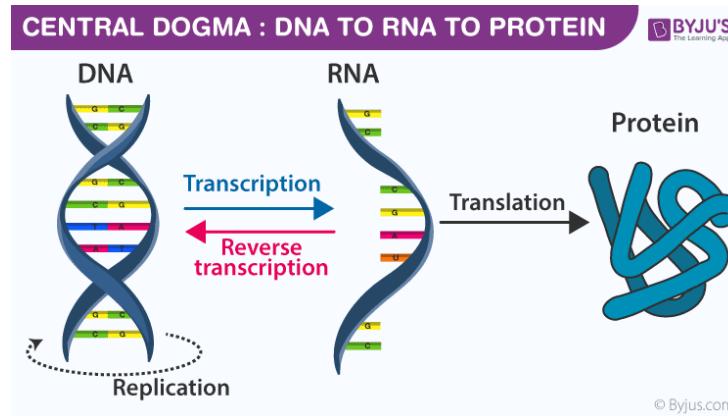
Your behaviors and environment can cause changes that affect the way your genes work



[Epigenetics - Wikipedia](#)

Genetics

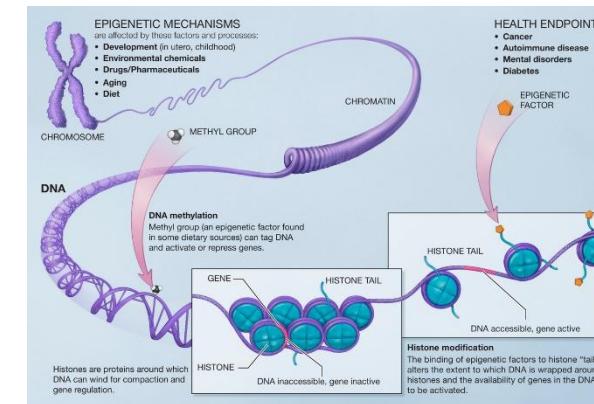
Your genes play an important role in your health



A woman without her man is nothing

Epigenetics

Your behaviors and environment can cause changes that affect the way your genes work



A woman, without her man, is nothing
A woman: without her, man is nothing

Genetics

“Reading the book of life”

EPIGENETIC REGULATION OF NORMAL CELLULAR PROCESSES IS TYPICALLY DRIVEN IN A CELL TYPE DEPENDENT MANNER. THIS REQUIRES AN EXQUISITE LEVEL OF INTERPLAY BETWEEN EPIGENETIC LAYERS INCLUDING DNA METHYLATION, NUCLEOSOME POSITIONS AND HISTONE MODIFICATIONS AMONGST OTHERS TOGETHER. THE EPIGENETIC MECHANISM ESTABLISHES THE CORRECT GENE EXPRESSION PATTERNS AND HIGHER ORDER CHROMATIN STRUCTURES. THUS, THE IDENTITY OF EACH CELL IS DETERMINED BY THE PATTERN OF DNA METHYLATION. THESE PATTERNS ARE ESTABLISHED DURING EMBRYONIC DEVELOPMENT AND ARE MITOTICALLY HERITABLE THROUGH MULTIPLE CELLULAR DIVISIONS.

Epigenetics

“Translating the book of life”

EpiGenetic regulation of normal cellular processes is typically driven in a cell type-dependent manner. This requires an exquisite level of interplay between epigenetic layers, including DNA methylation, nucleosome positions and histone modifications, amongst others.

Together, epigenetic mechanisms establish the correct gene-expression patterns and higher-order chromatin structures; thus, the identity of each cell. Of these, DNA methylation is the best-studied epigenetic modification. Precise DNA methylation patterns are established during embryonic development and are mitotically heritable through multiple cellular divisions.



Genetics



The structure of DNA was discovered in 1953

```
GTAAGGCAGTCGATATAGGTT  
CACCGGTCTTAGGCACGTTGAG  
CTGACCAGTGCTAACGTCCAA  
TGGCATTGCAGCTGGGGTACAC  
AGTCTTGTGTCACAGTCAGGTC  
TATCTGTACTAGTCTTACGTAGT  
CGGTACCCCATGACATCGACTT  
GTGTCGGATCGATCATC
```

DNA code was mapped in 2001 – 3 billion letters (GATC)

Genetics



How does each cell know what it is supposed to be?

```
GTAAGGCAGTCGATATAGGTT  
CACCGGTCTTAGGCACGTTGAG  
CTGACCAGTGCTAACGTCAA  
TGGCATTGCAGCTGGGGTACAC  
AGTCTTGTGTCACAGTCAGGTC  
TATCTGTACTAGTCTTACGTAGT  
CGGTACCCCATGACATCGACTT  
GTGTCGGATCGATCATC
```

DNA code was mapped in 2001
– 3 billion letters (GATC)

Genetics



How does each cell know what it is supposed to be?

Epigenetics



```
GTAAGGCAGTCGATATAGGTT  
TTGAG  
CTGACCAGTGCTAACGTCCAA  
TGGCATTGCAGCTGGGGTACAC  
AGTCTTGTGT  
  
CGGTACCCCATGACATCGACTT  
GTGTCGGATCGATCATC
```

Only parts of the genome are active at any one time

COL1A1 gene expressed in hair cells, but not brain cells

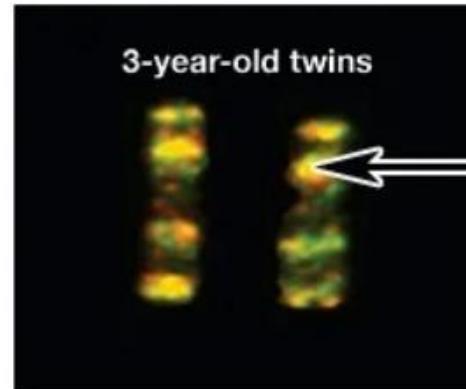
Example 1: Identical twins

Identical twins come from the same fertilized egg
→ share identical genetic profiles (genome)

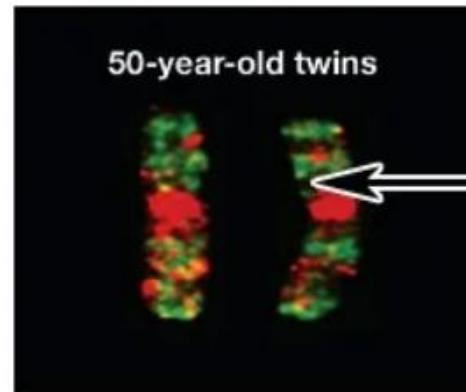


→ Your behaviors and environment can cause changes that affect the way your genes work

Chromosome 3 Pairs
3-year old twins vs. 50-year-old twins



Yellow shows where the twins have epigenetic tags in the same place.



Red and green show where the twins have epigenetic tags in different places.

Chromosome 3 pairs in each set of twins are digitally superimposed. One twin's epigenetic tags are dyed red and the other twin's tags are dyed green. When red and green overlap, that region shows up as yellow. The 50-year old twins have more epigenetic tags in different places than do 3-year-old twins.

Example 2: Identical twins 2

Identical twins come from the same fertilized egg → share identical genetic profiles (genome)

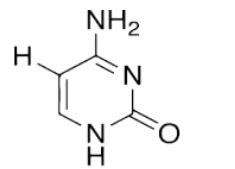


Leora Eisen

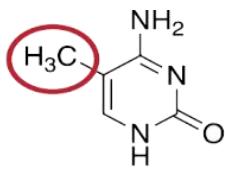
Linda Lewis
(leukemia)

→ Your behaviors and environment can cause changes that affect the way your genes work

What are DNA Methylation and genome-wide DNA methylation (Epigenome)?

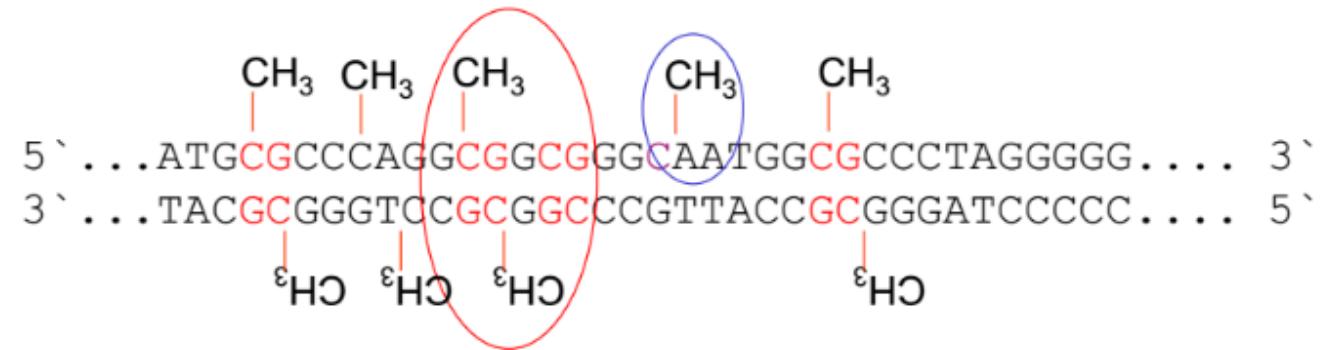
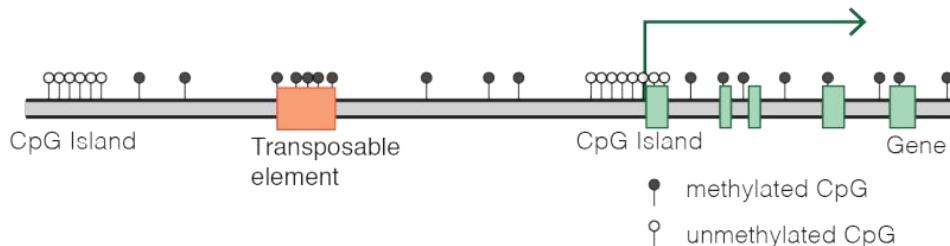


Cytosine

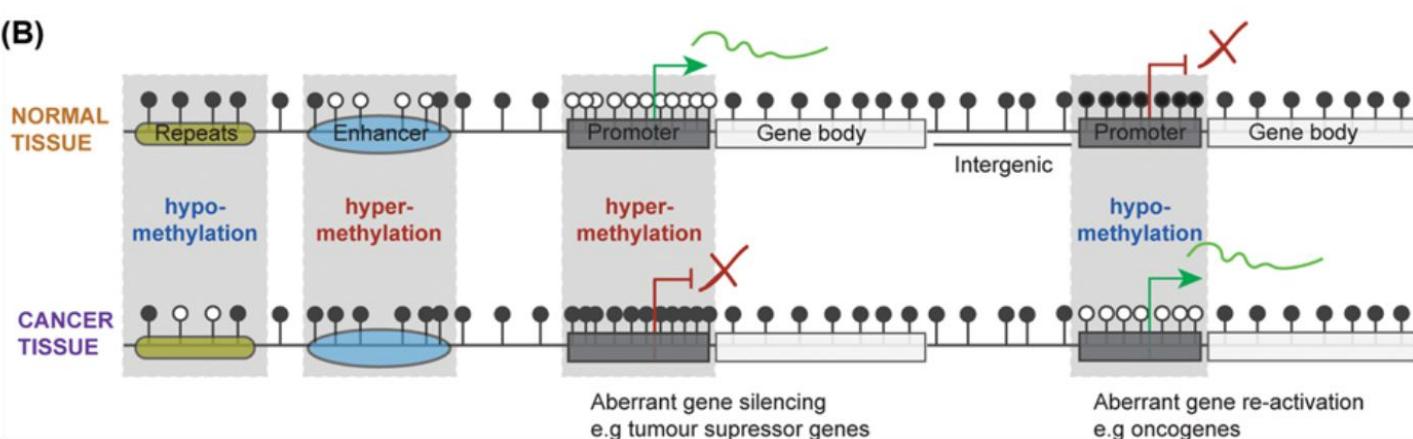


methylated Cytosine

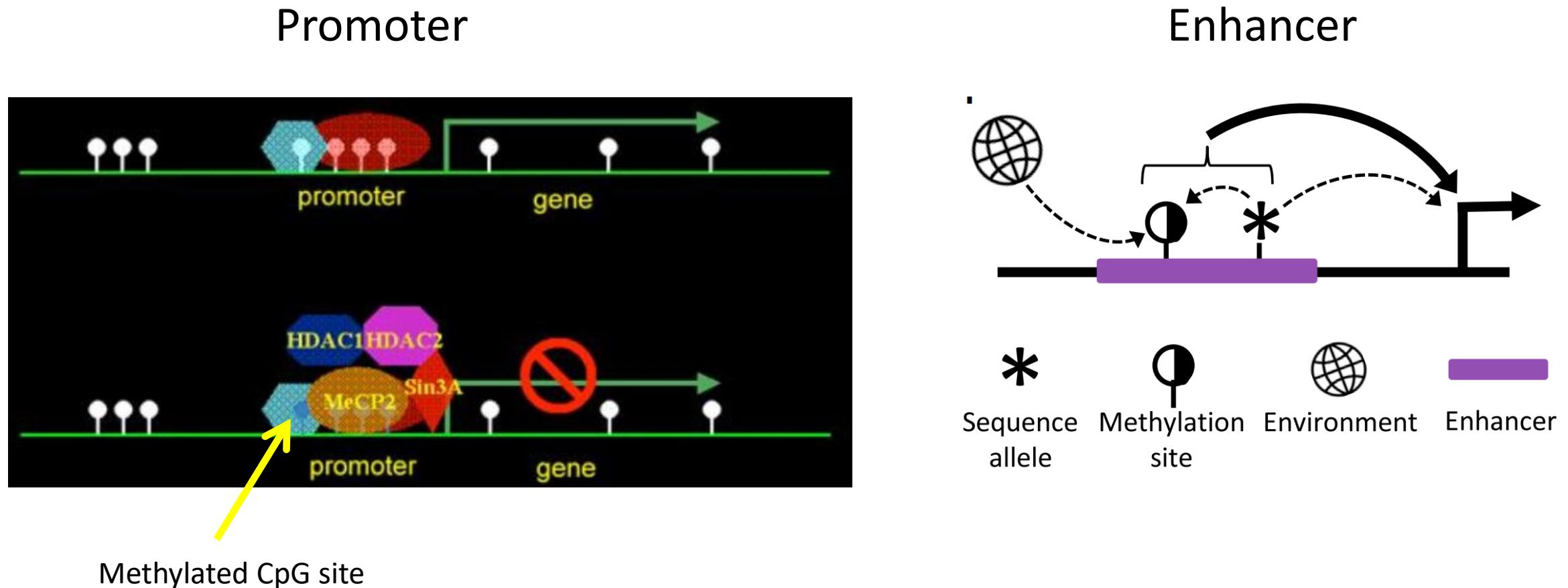
Typical mammalian DNA methylation landscape



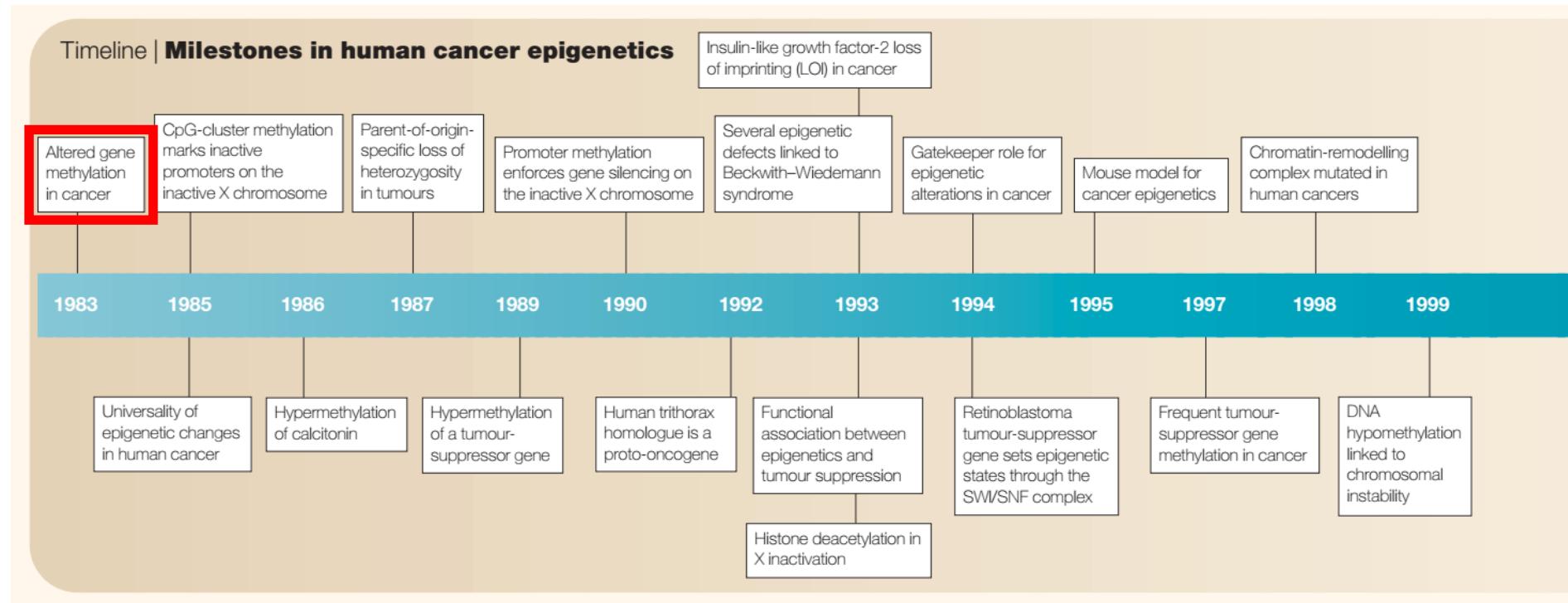
(B)



DNA methylation regulates gene expression

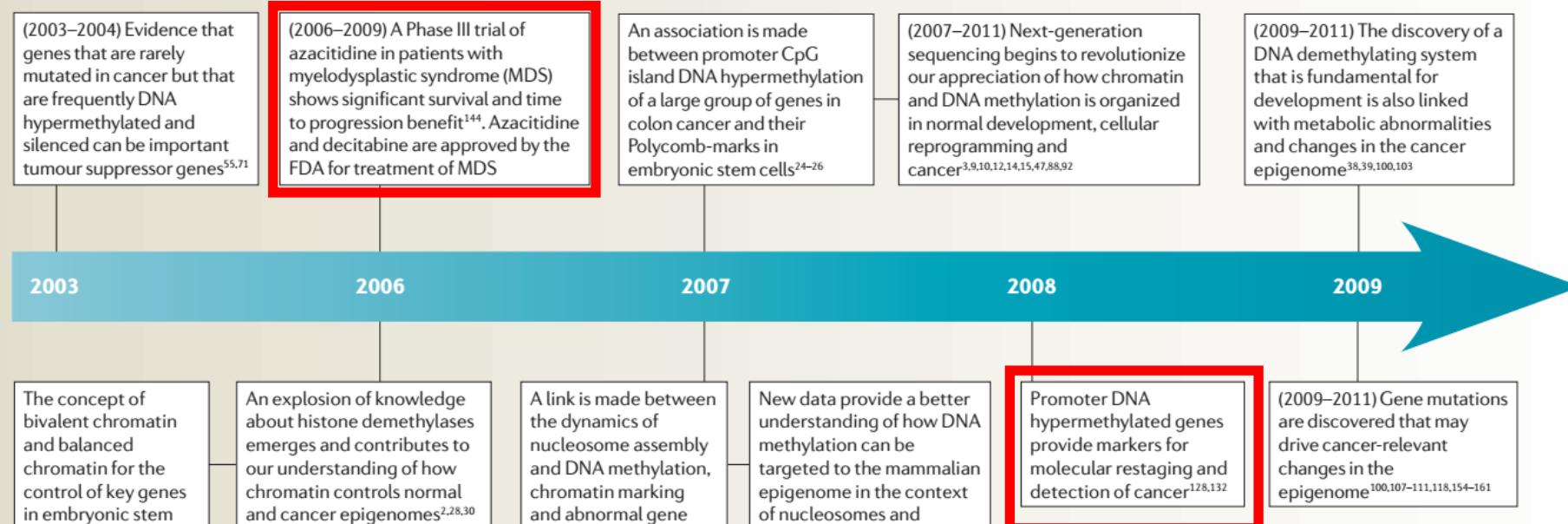


DNA methylation regulate gene expression in cancer



DNA methylation regulate gene expression in cancer

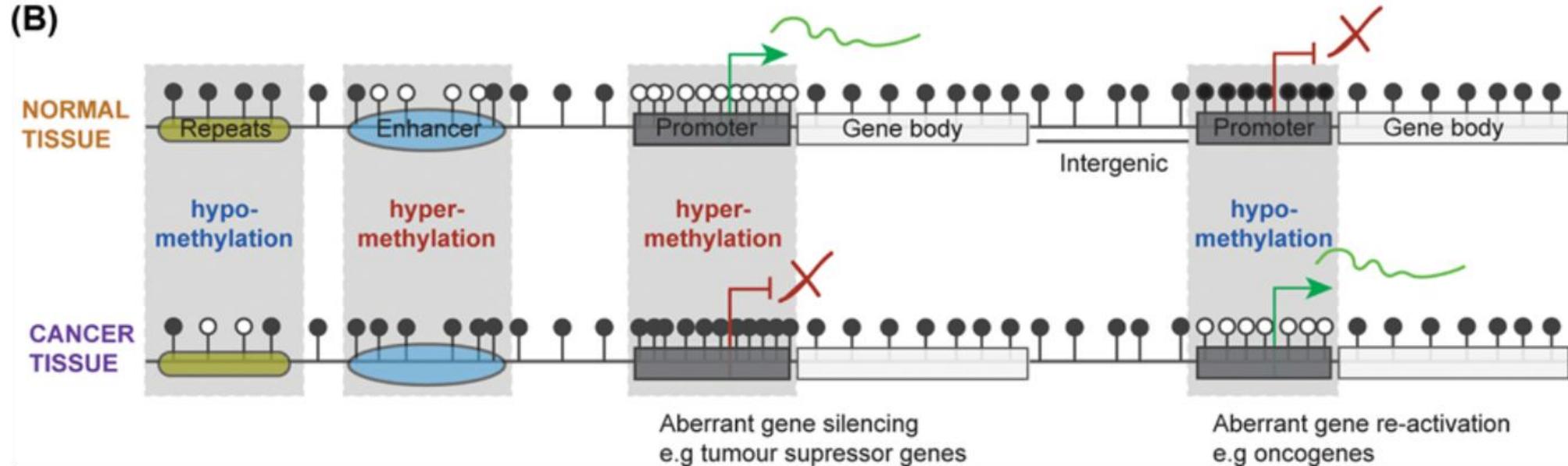
Timeline | Examples of key advances in epigenetics and cancer over the past decade



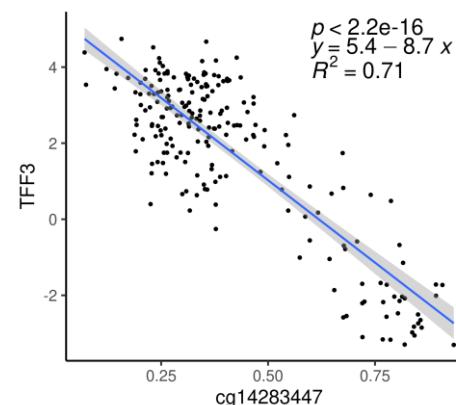
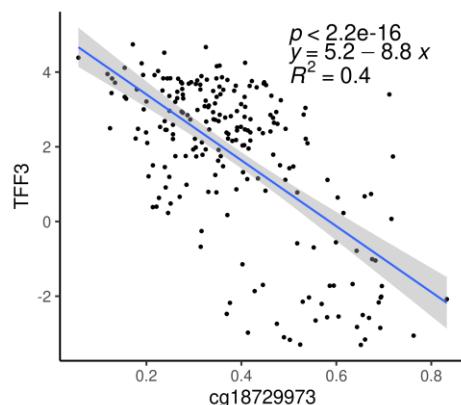
FDA, US Food and Drug Administration.

DNA methylation regulate gene expression in cancer

(B)



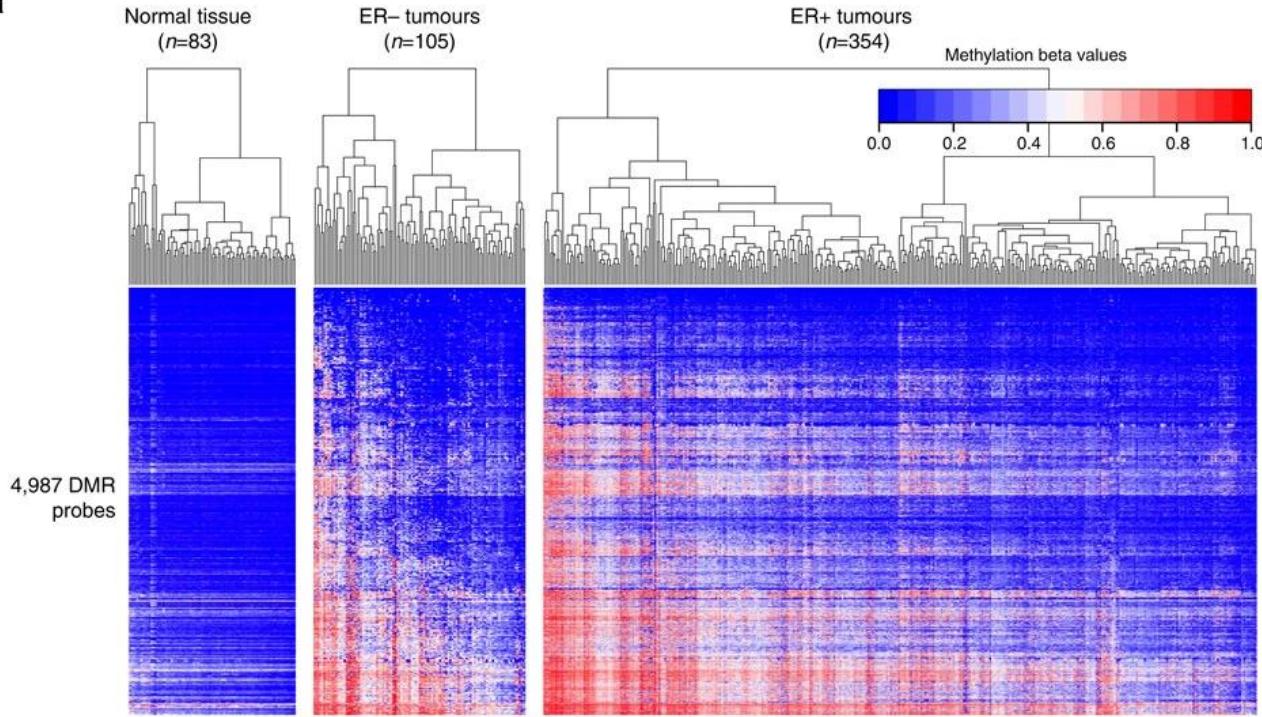
Expression



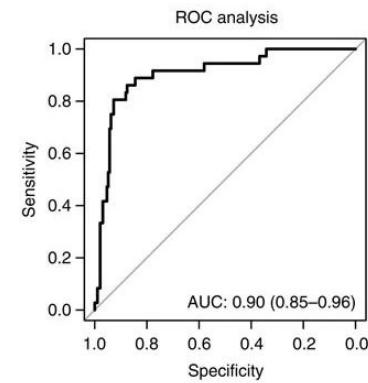
DNA methylation

DNA methylation regulate gene expression in breast cancer

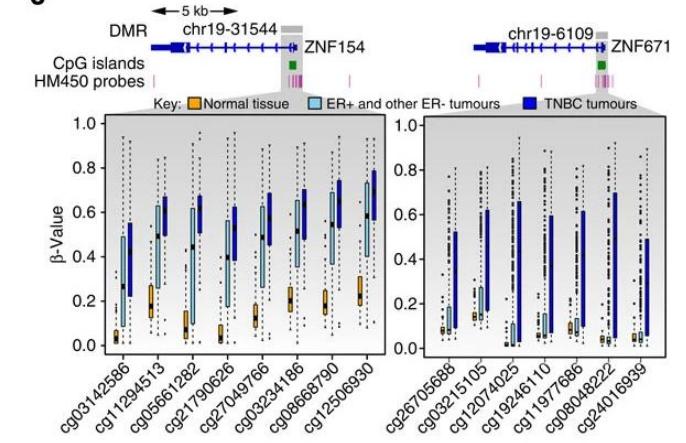
a



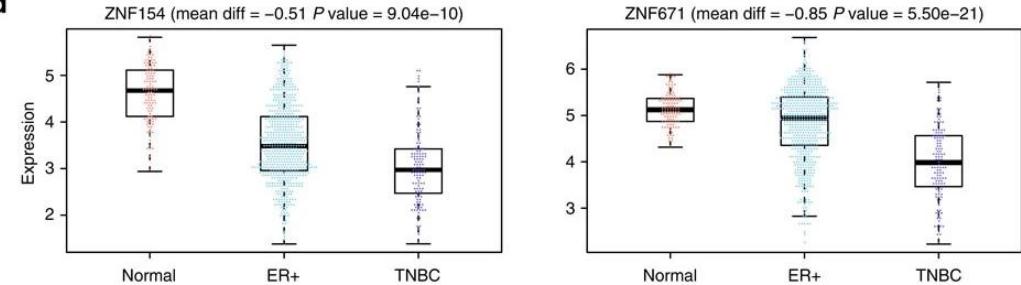
b



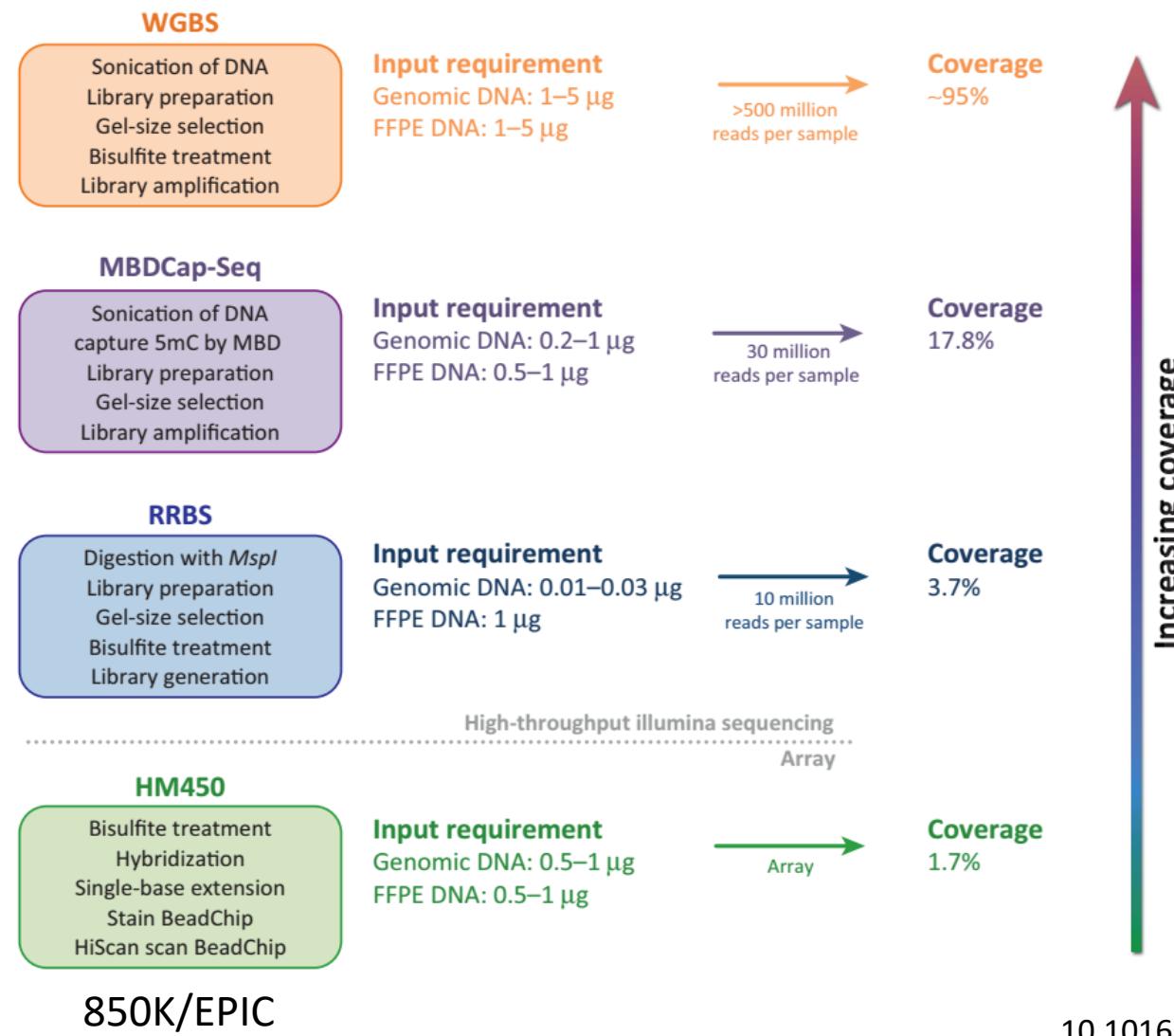
c



d



Methods to profile DNA methylation



Bisulphite Sequencing

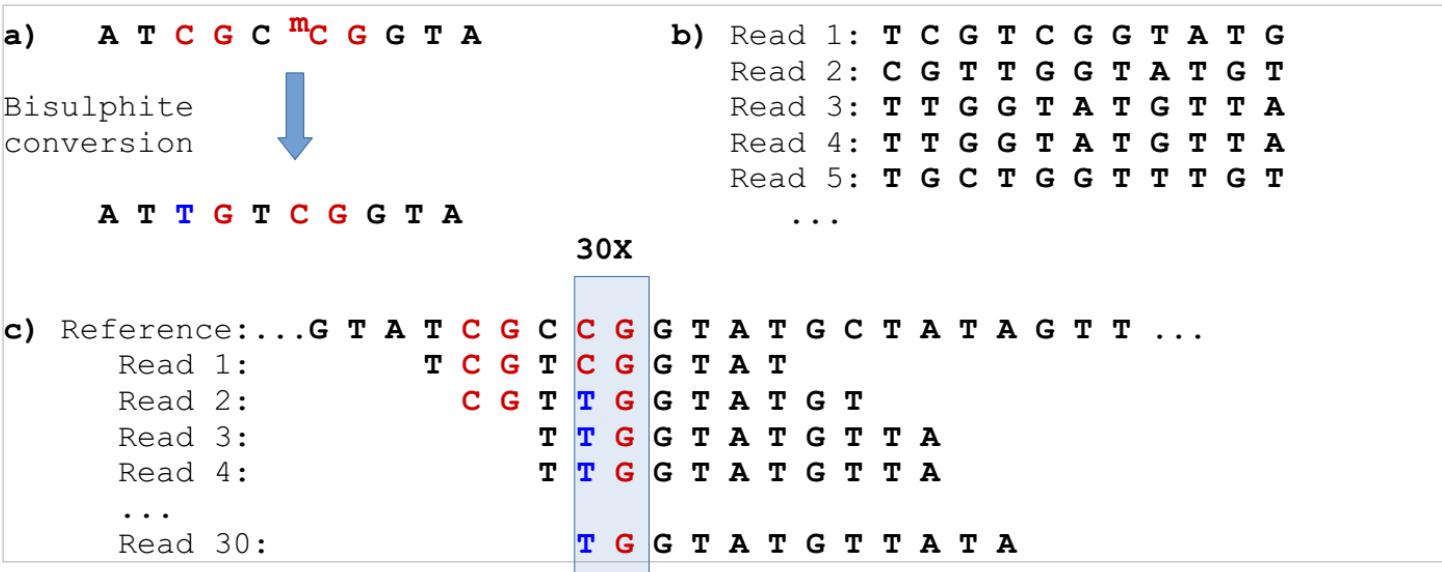


Figure 1. a) Sodium bisulphite converts unmethylated cytosines to thymines. b) The converted DNA is sequenced. c) Alignment and aggregation of these short sequence can be used to quantify the ratio of methylated (C) to unmethylated (converted T) cytosines from a population of cells at a single locus.

$$\begin{aligned}\text{Beta value} &= \text{Per} \\ \text{Per (1)} &= 100 * M / \text{Cov (2)} \\ \Rightarrow M &= \text{Per} * \text{Cov} / 100 \\ \Rightarrow \text{Cov} &= C + T\end{aligned}$$

Total_cov_matrix_CpG.txt

	A	B	C	D
1	chr	pos	nmDNA1	nmDNA2
2	chr2	133031228	56	55
3	chr2	133031240	74	81
4	chr2	133031244	82	79
5	chr2	133031249	64	70
6	chr2	133031265	65	69
7	chr2	133031268	67	56

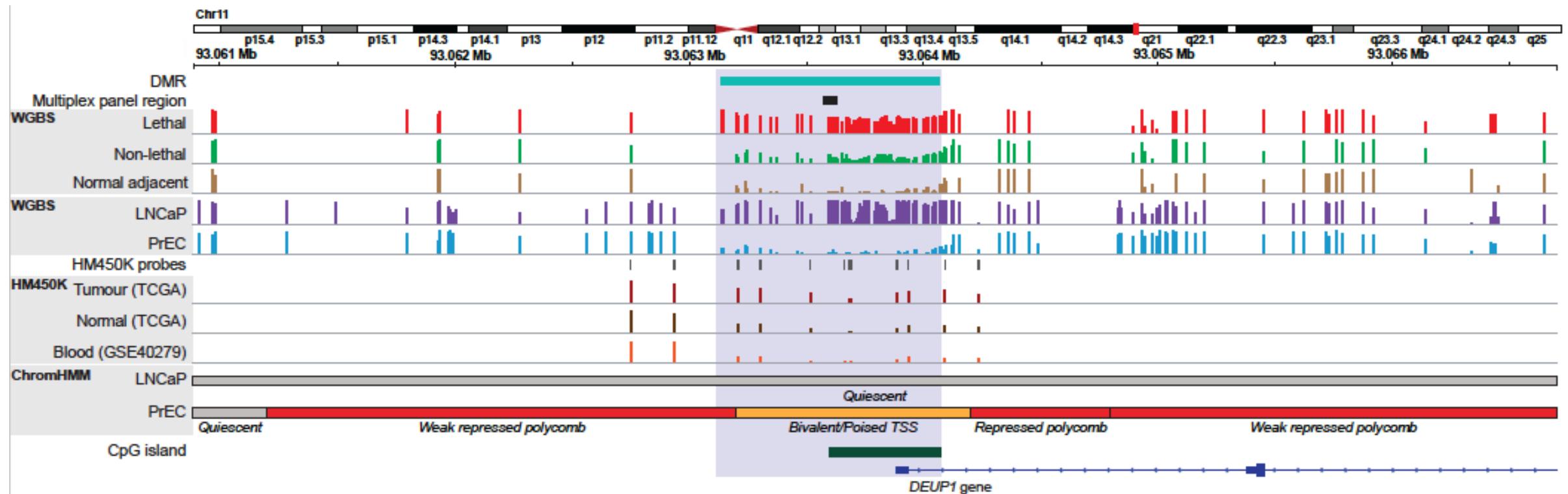
Meth_cov_matrix_CpG.txt

	A	B	C	D
1	chr	pos	nmDNA1	nmDNA2
2	chr2	133031228	41	28
3	chr2	133031240	29	19
4	chr2	133031244	47	47
5	chr2	133031249	35	24
6	chr2	133031265	19	21
7	chr2	133031268	24	16

Perc_cov_matrix_CpG.txt

	A	B	C	D
1	chr	pos	nmDNA1	nmDNA2
2	chr2	133031228	73	51
3	chr2	133031240	39	23
4	chr2	133031244	57	59
5	chr2	133031249	55	34
6	chr2	133031265	29	30
7	chr2	133031268	36	29

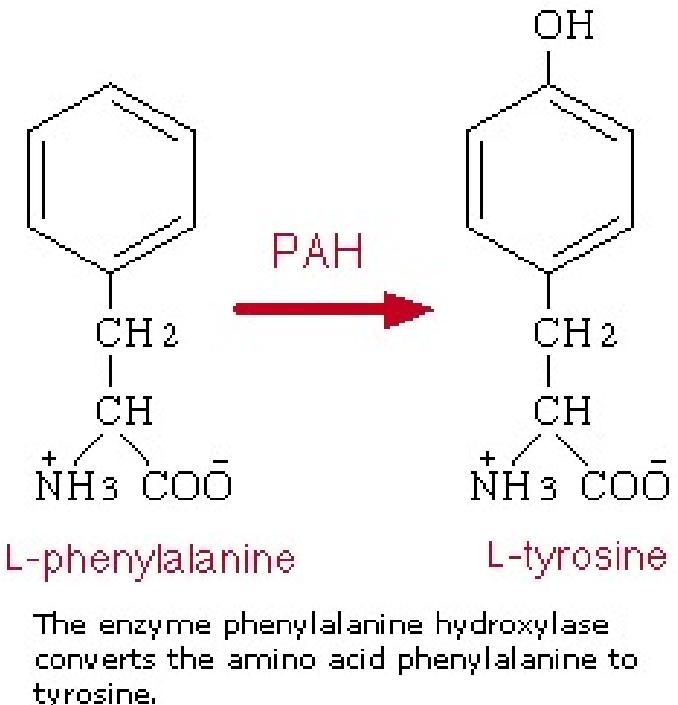
WGBS vs 450K



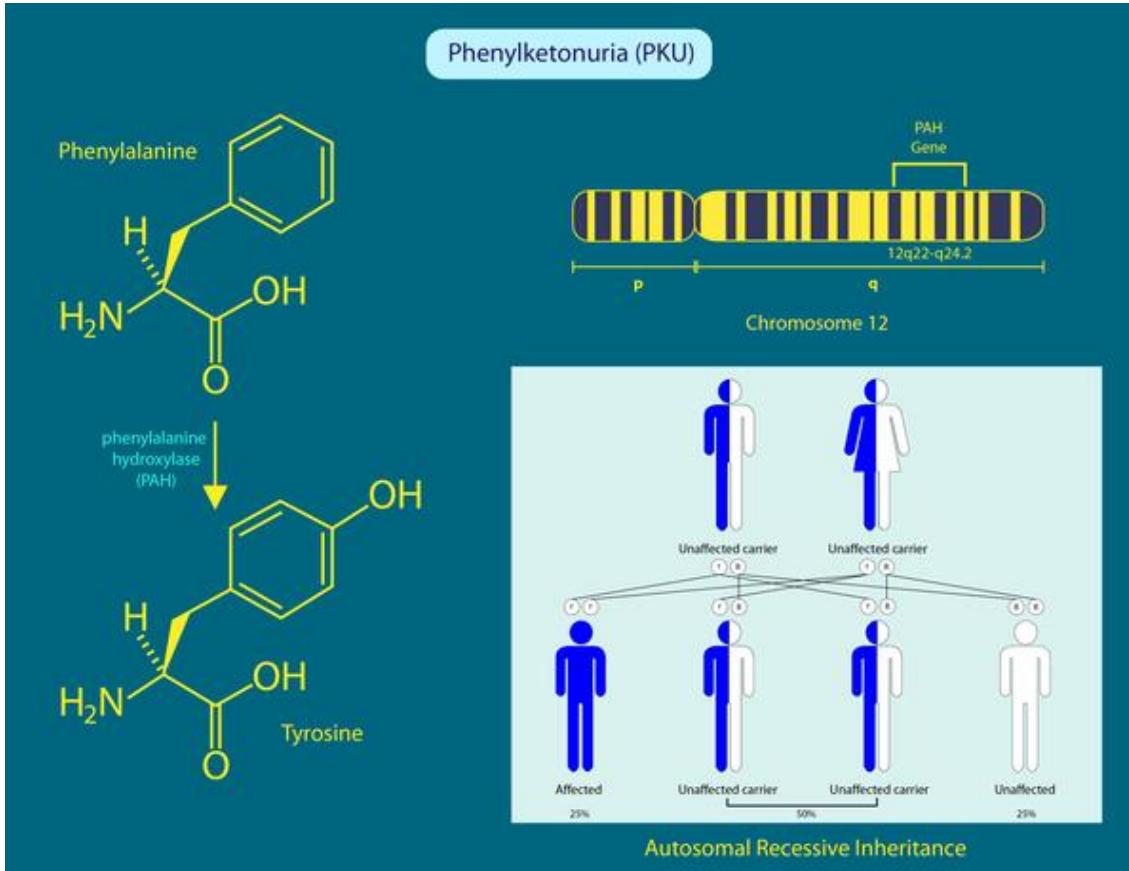
Ví dụ mối quan hệ của
BIẾN THỂ GEN và **BỆNH DI TRUYỀN**

Phenylketonuria (PKU): Rối loạn chuyển hóa di truyền

- Nguyên nhân do thiếu hụt enzyme phenylalanine hydroxylase.
- Sự mất enzyme này dẫn đến suy giảm trí tuệ, tổn thương cơ quan, tư thế bất thường.
- Tần suất xảy ra PKU khác nhau giữa các nhóm dân tộc và các vùng địa lý trên toàn thế giới. Ở Hoa Kỳ, PKU xảy ra ở 1 trong 25.000 trẻ sơ sinh.
- Hầu hết các trường hợp PKU được phát hiện ngay sau sinh bằng sàng lọc sơ sinh và điều trị được bắt đầu ngay lập tức.



Phenylketonuria (PKU): Rối loạn chuyển hóa di truyền



Tên khác của PKU

- Folling disease
- Folling's disease
- PAH deficiency
- Phenylalanine hydroxylase deficiency
- Phenylalanine hydroxylase deficiency disease

Trình tự gen PAH ở người - Homo sapiens (5053)

Gene (Nucleotide)

NT seq	1359 nt NT seq atgtccactgcggctggaaaacccaggctggcaggaaactctctgactttggacag gaaacaagcttatattgaagacaactgaatcaaaatggccatatcactgatcttca ctcaaagaagaagttggcattggccaaagtattgcgttatttggaggagaatgtat aacctgaccacattgaatcttagacccctcgtttaaagaaaagatgagttatgaaat accctattggataaaacgttagcctgcgtctgacaaaatcatcaagatcttgggcat gacattggtggccactgtccatgagcttcacggataagaagaaaagacacagtggccctgg ttcccaagaaccattcaagagctggacagatggccaatcagattctcgtatggagcg gaactggatgctgaccaccctggtttaaagatccgtgtaccgtgcaagacggaaagcg tttgcgtacattgcctacaactaccggcatgggcagccatccctcgagtggaaatacatg gaggaagaaaaagaaaatggggcacatgttcaagactctgaaagtccctgtataaaaacc catgttgcgtatgagttacaatcacatccacttgcgttgcagacttgcactggccat gaagataacatccccagctggaaagacgttctcgttgcagacttgcactggccat cgccctccgacactgtggctggcctgtccctctcgggatttctggggccat cgagtctccactgcacacatcagatggatccaagccatgtataccccgaa cctgacatctgcatgagctgtggacatgtgcccttgcgttgcagatgcgcacttgc cagtttccaggaaatggccctgcctctgggtgcacatgtgaaatacatgtaaaag ctcgccacaatgttactgtggatgttgcgtctgcaaaacaggagactccata aaggcatatggtgcggccctgtcatccttggtaattacagactgttgcattatcagag aagccaaagcttctcccccggagctggagaagacagccatccaaaattacactgtcag gagttccagccctcttacatgtggcagagatgttgcacatgtgcaaggagaaaatgt aactttgcgtccacaatccgtggcccttcgttgcgtacgacccatcacccaaagg attggggatcttgcgtggcctccaggaaaatgttgcgttgcattaaacatgtgaa attggaaatcttgcgtggcctccaggaaaatgttgcgttgcattaaacatgtgaa
--------	--

Protein (Amino Acid)

AA seq	452 aa AA seq DB search MSTAVLENPGLGRKLSDFGQETSYIEDNCNQNGAISLIFSLKEEVGALAKVLRLFEENDV NLTHIESRPSRLKKDEYEFFTHLDKRSLPALTNIKILRHDIGATVHELSRDKKKDTVPW FPRTIQELDRFANQILSYGAELDADHPGFKDPMVYRARRKQFADIAYNYRHGQPIPRVEYM EEEKKTWGTVFKTLKSLYKTHACYEYNHIFPLLEKYCFGHEDNIPQLEDVSQFLQTCTGF RLRPVAGLLSSRDFLGLAFRVFHCTQYIRHGSKPMTPEPDICHELLGHVPLFSDRSFA QFSQEIGLASLGAPDEYIEKLATIYWFTVEFLCKQGDSIKAYGAGLLSSFGELQYCLSE KPKLLPLELEKTAIQNYTVTEFQPLYYVAESFNDAKEKVRNFAATIPRPFNSVRYDPYTQR IEVLDNTQQLKILADSINSEIGILCSALQKIK
--------	---

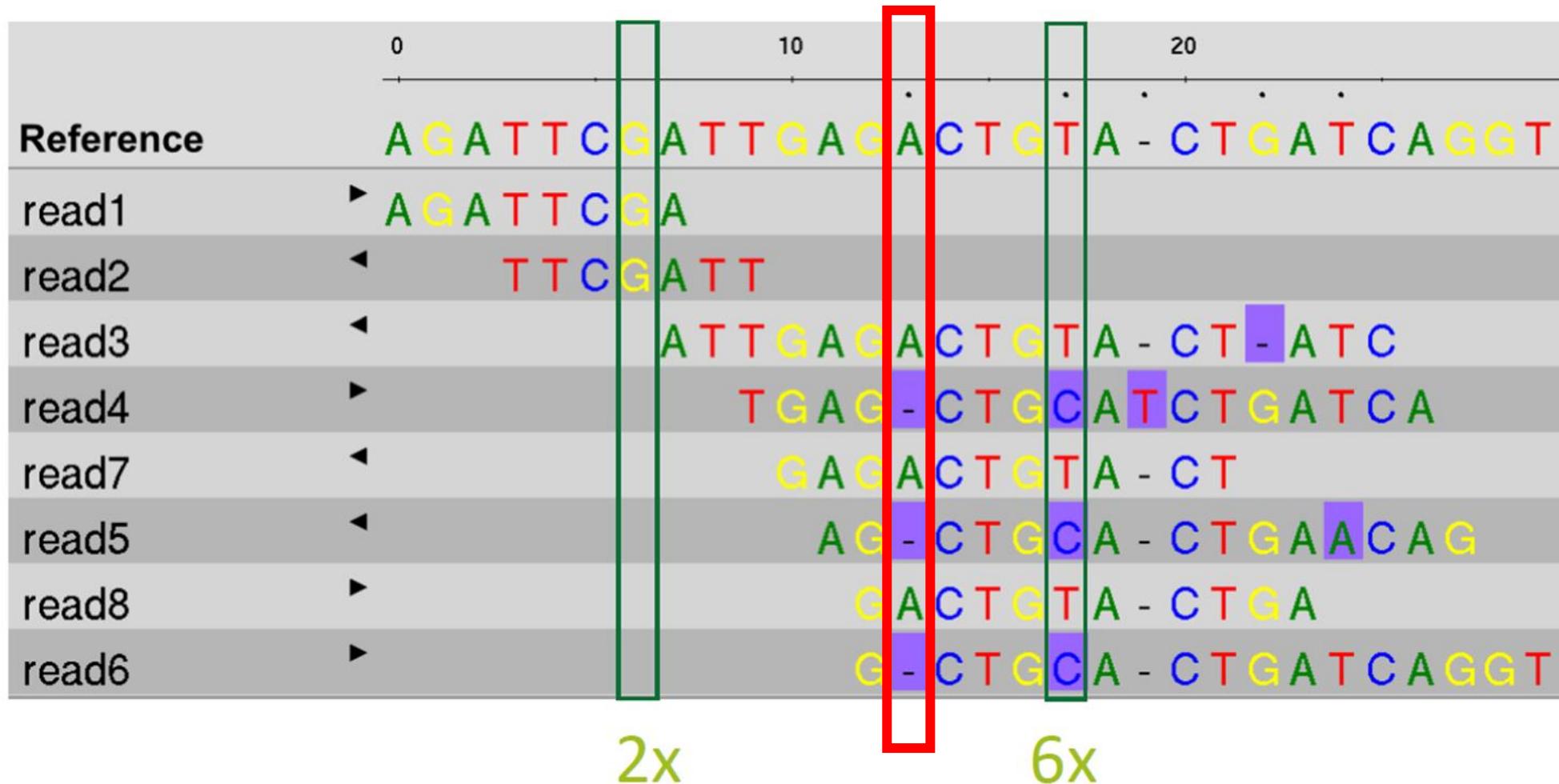
<https://www.genome.jp/entry/T01001:5053>

Gen PAH



<https://www.ncbi.nlm.nih.gov/gene/5053>

Variants in PAH



Cơ sở dữ liệu ClinVar cho gen PAH

Classification type

- Germline (208)
- Somatic (0)

Germline classification

- Conflicting classifications (2)
- Benign (10)
- Likely benign (21)
- Uncertain significance (41)
- Likely pathogenic (40)
- Pathogenic (39)

Types of conflicts

- P/LP vs LB/B (0)
- P/LP vs VUS (0)
- VUS vs LB/B (2)

Molecular consequence

- Frameshift (20)
- Missense (96)
- Nonsense (7)
- Splice site (9)
- ncRNA (0)
- Near gene (0)
- UTR (27)

Variation type

- Deletion (39)

Links from Gene

[Display options](#) ▾ [Sort by Relevance](#) ▾ [Download](#) ▾

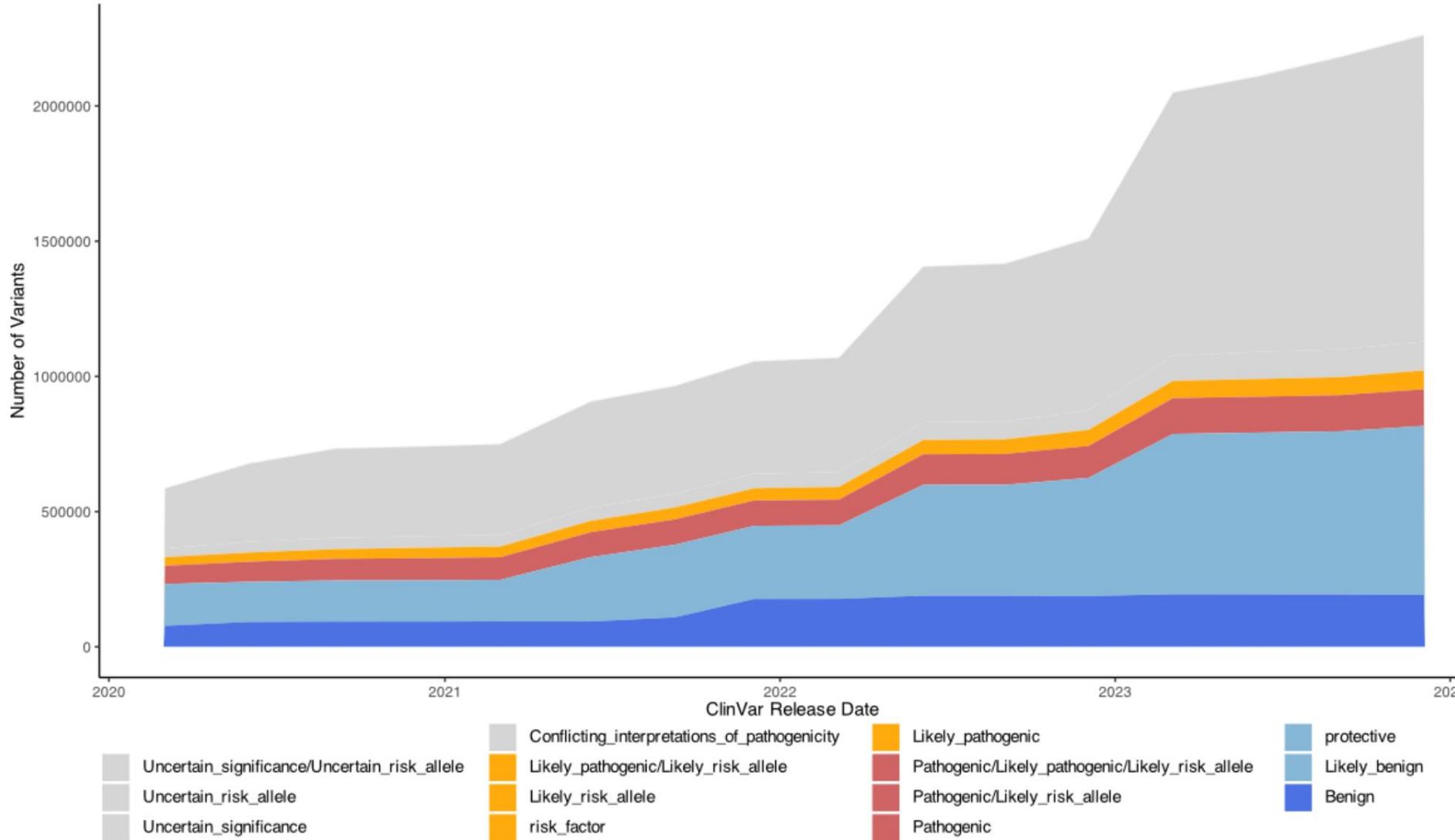
Items: 1 to 100 of 209

<< First < Prev Page of 3 Next > Last >>

Variation	Gene (Protein Change)	Type (Consequence)	Condition	Classification, Review status
<input type="checkbox"/> NM_004316.4(ASCL1):c.51G>T (p.Gln17His)	ASCL1, PAH (Q17H)	Single nucleotide variant (missense variant +1 more)	not specified	 Uncertain significance ★
<input type="checkbox"/> NC_000012.11:g.(?_103232953)_(1_03240749_?)del	PAH	Deletion	Phenylketonuria	 Pathogenic ★
<input type="checkbox"/> NC_000012.11:g.(?_103288493)_(1_03310908_?)del	PAH	Deletion	Phenylketonuria	 Pathogenic ★
<input type="checkbox"/> NC_000012.11:g.(?_103248894)_(1_03249131_?)del	PAH	Deletion	Phenylketonuria	 Pathogenic ★
<input type="checkbox"/> NC_000012.12:g.(?_102894715)_(102894938_?)del	PAH	Deletion	Phenylketonuria	 Pathogenic ★
<input type="checkbox"/> NC_000012.11:g.(?_103306549)_(1_03306696_?)del	PAH	Deletion	Phenylketonuria	 Pathogenic ★
<input type="checkbox"/> NM_000277.3(PAH):c.1179_1180del (p.Asn393fs)	PAH (N393fs)	Deletion (frameshift variant)	Phenylketonuria	 Likely pathogenic ★

https://www.ncbi.nlm.nih.gov/clinvar?LinkName=gene_clinvar&from_uid=5053

Overview of ClinVar release trends over time



The classes have been divided into five colors to represent the degree of pathogenicity, from blue (benign) to red (pathogenic) with gray representing variants that are of uncertain clinical significance or have no clinical significance.

Biến thể gây bệnh - Pathogenic variant in PAH

NM_000277.3(PAH):c.971T>A (p.Ile324Asn)

ClinVar Genomic variation as it relates to human health

Search by gene symbols, location, HGVS expressions, c-dot, p-dot, conditions, : **Search ClinVar** ?

[Advanced search](#)

[About](#) [Access](#) [Submit](#) [Stats](#) [FTP](#) [Help](#) Like Dislike

NM_000277.3(PAH):c.971T>A (p.Ile324Asn) [Cite](#) [Follow](#) [Print](#) [Download](#)

We've updated the ClinVar website to better support classifications of somatic variants!

Read more about changes to the website in our [web release notes](#); more information about somatic variants in ClinVar is available on [GitHub](#).

Germline

Top reviewed classifications are shown here. Submission summary: **1 submission 1 submitter 1 condition**

Reviewed by expert panel **Pathogenic** for Phenylketonuria

Dec 2023 by [ClinGen PAH Va...](#) [FDA RECOGNIZED DATABASE](#)

Somatic

No data submitted for somatic clinical impact

No data submitted for oncogenicity

On this page

[Classification Summary](#) [Variant Details](#) [Genes](#) [Germline](#) [Conditions](#) [Submissions](#) [Citations](#) [Text mined Citations](#)

Feedback

<https://www.ncbi.nlm.nih.gov/clinvar/variation/2682170/>

Các biến thể gây bệnh trên gen PAH

Gene: PAH

[View on UniProt](#)

[View on SwissModel](#)

Transcript: ENST00000553106.6

Select protein structure

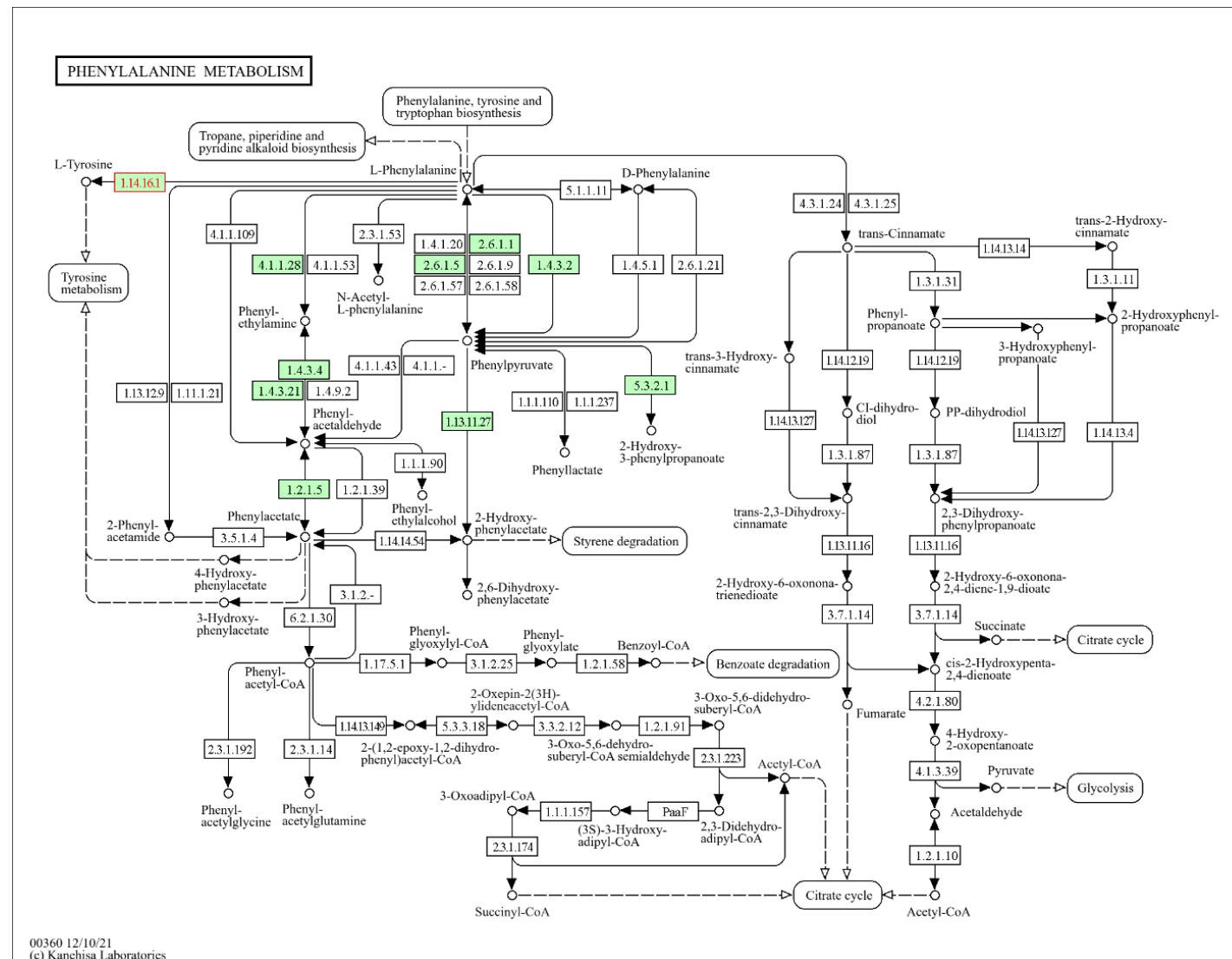
SwissModel:5den 20-450 (number o...)

Show

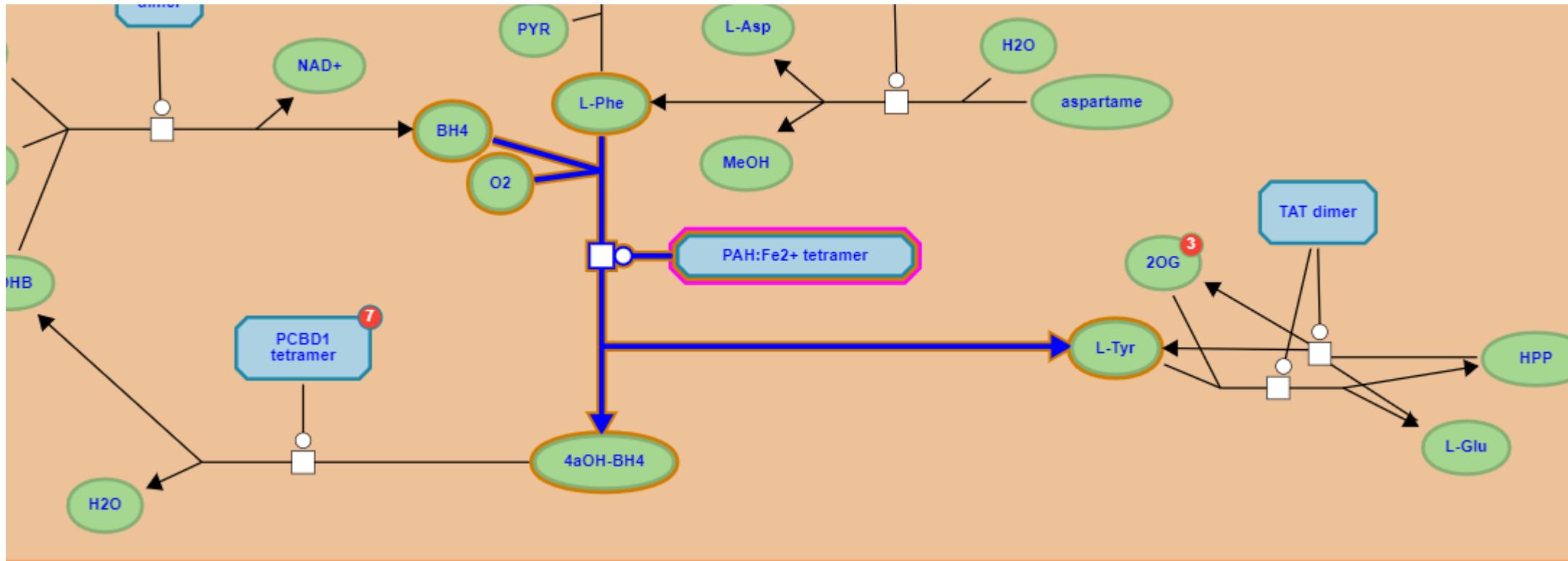
- All Residues
- Variants
- Pathogenic
- Likely Pathogenic
- Uncertain Significance
- Likely Benign
- Benign
- Current Variant

<https://varsome.com/variant/hg38/chr12%3A102844430%3AA%3AT?>

PAH: chuyển hóa Phenylalanine thành Tyrosine

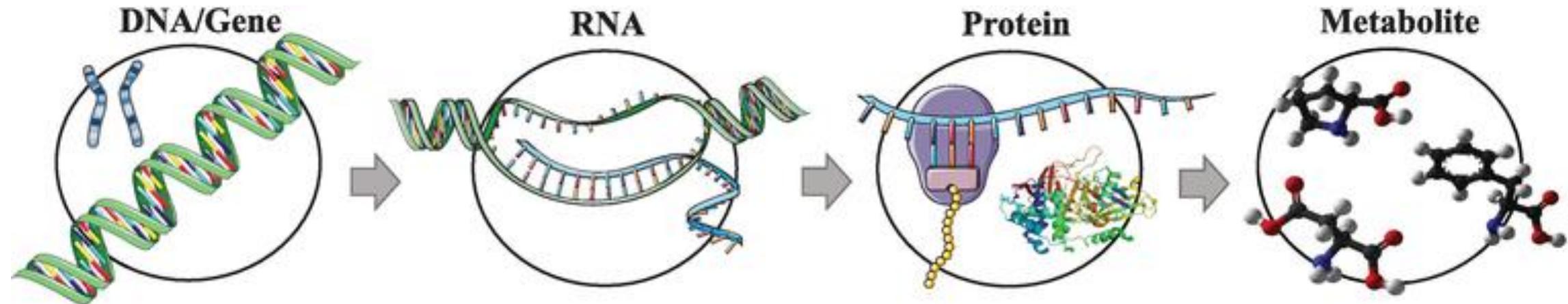


PAH: chuyển hóa Phenylalanine thành Tyrosine



<https://reactome.org/PathwayBrowser/#/R-HSA-8963691&SEL=R-HSA-71118&PATH=R-HSA-1430728,R-HSA-71291&FLG=UniProt:P00439>

Mối liên kết: Biến thể gen và bệnh di truyền



Genomics

PAH gene
Ref ...ATCGAT...
P1 ...AACGAT...
NM_000277.3(PAH):c.971T>A

Transcriptomics

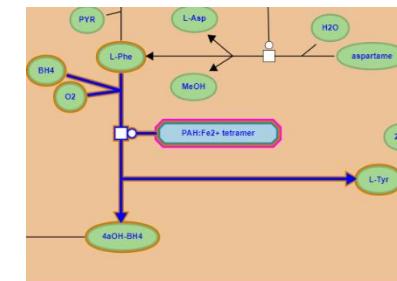
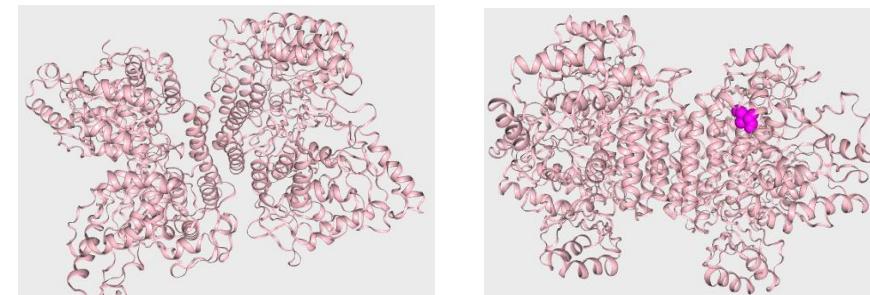
PAH mRNA
Ref ...AUCGAU...
P1 ...AACGAU...
NM_000277.3(PAH):c.971T>A

Proteomics

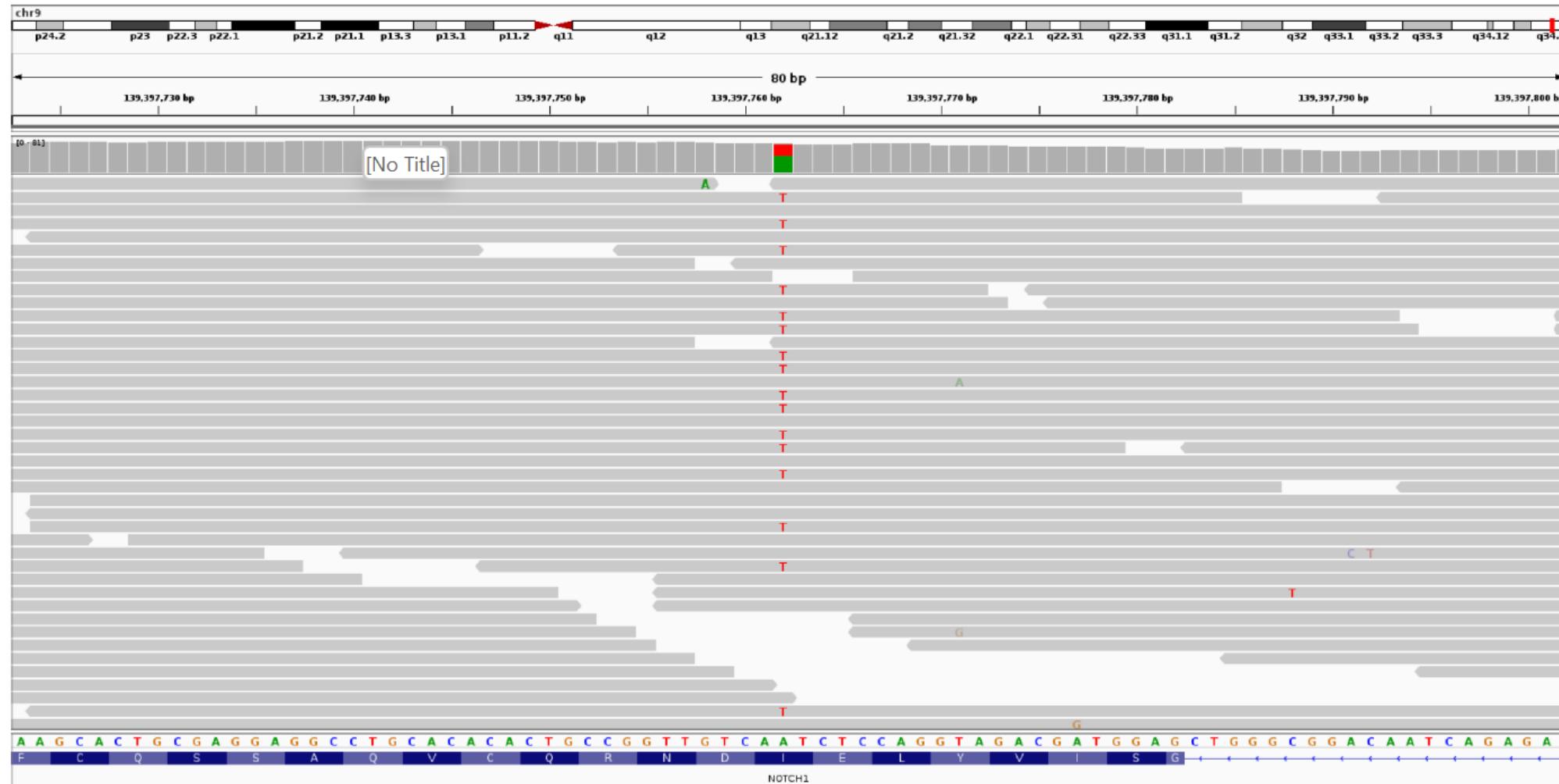
PAH protein
Ref ...Ile-Asp...
P1 ...Asn-Asp...
NM_000277.3(PAH):p.Ile324Asn

Metabolomics

PAH
Ref Phe → Tyr
PAH
P1 Phe ~~→~~ Tyr

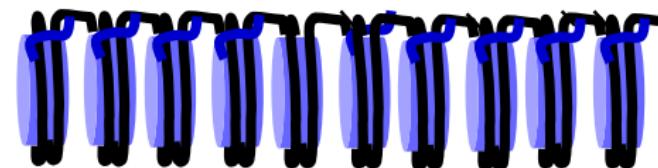


Alignment and variant viewers



Epigenetic control through chromatin regulation: The main players.....

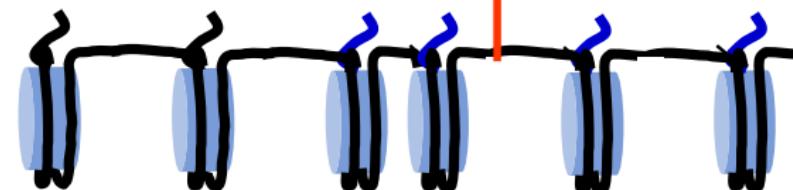
Closed, inaccessible chromatin



DNA-methylation

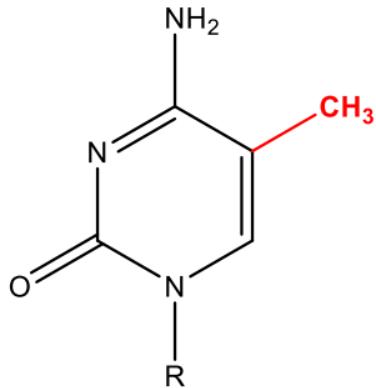
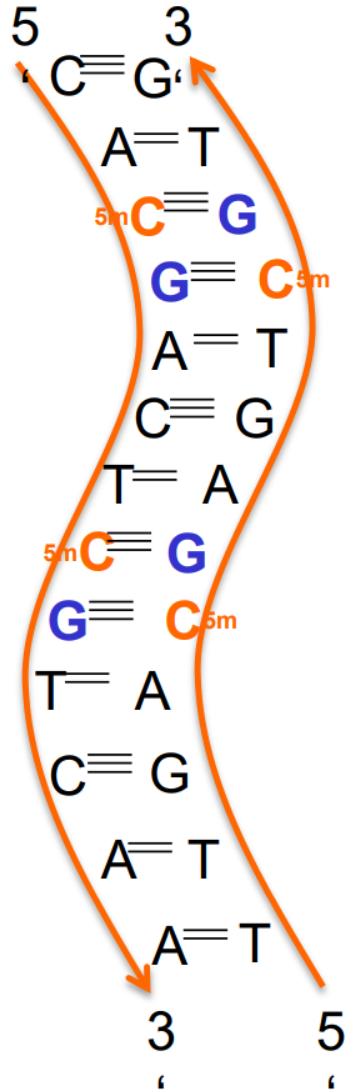
ncRNAs

Histone
Modifications



Open, transcriptionally competent chromatin

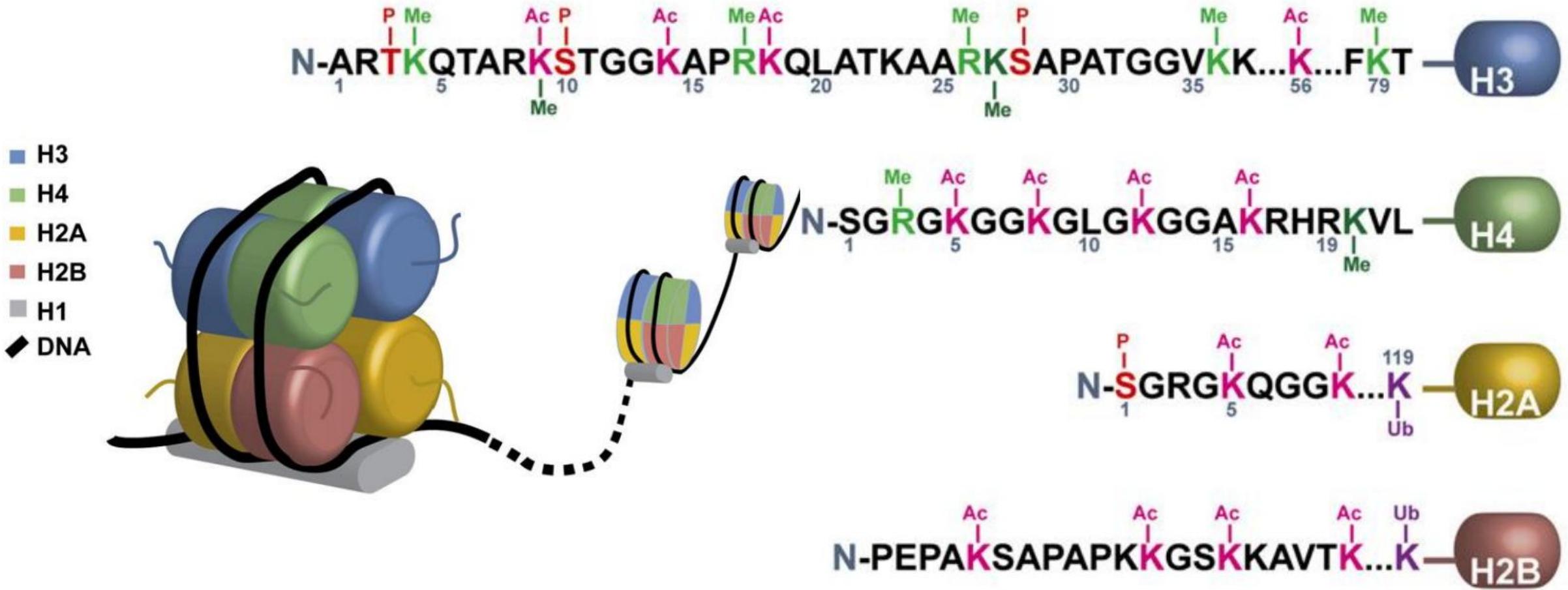
DNA-methylation



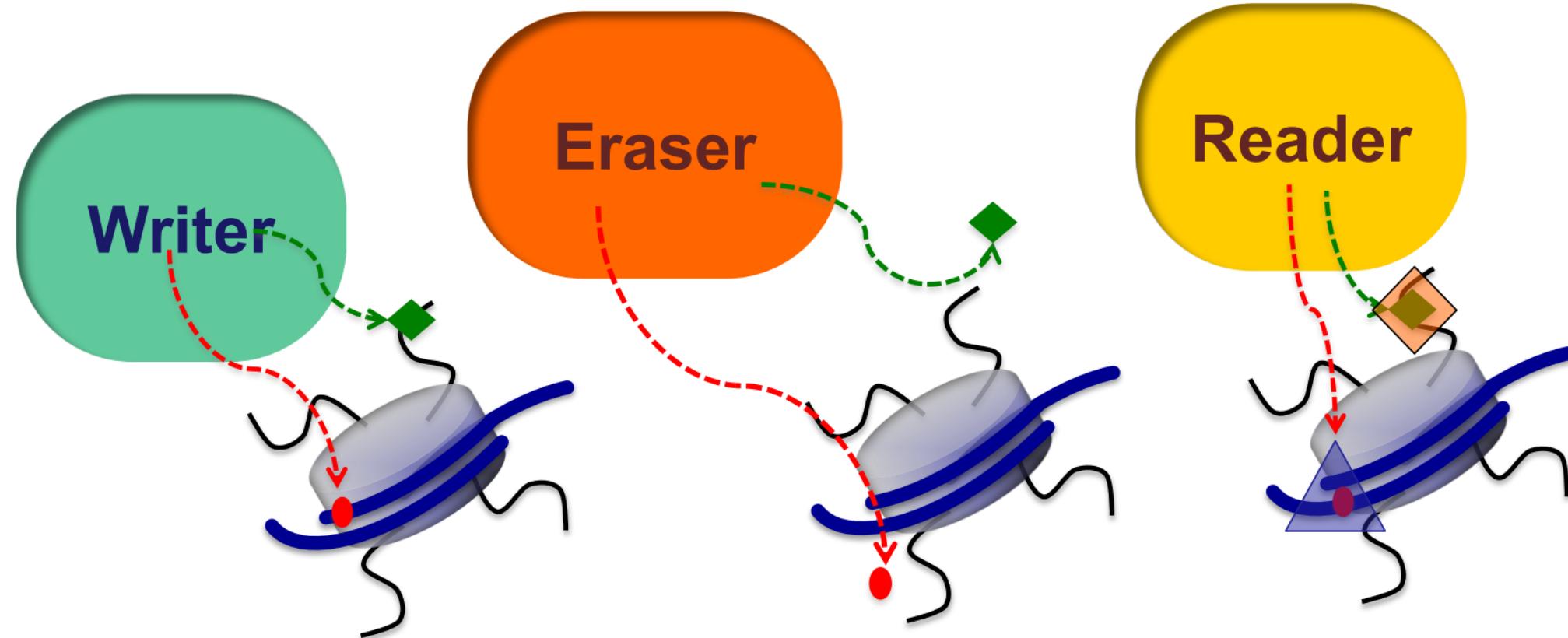
5-Methylcytosine (5mC)

DNA-Methylation is established
„postreplicative“ by
DNA-Methyltransferases (DNMT's)

Epigenetic modifications at the N-termini of Histones

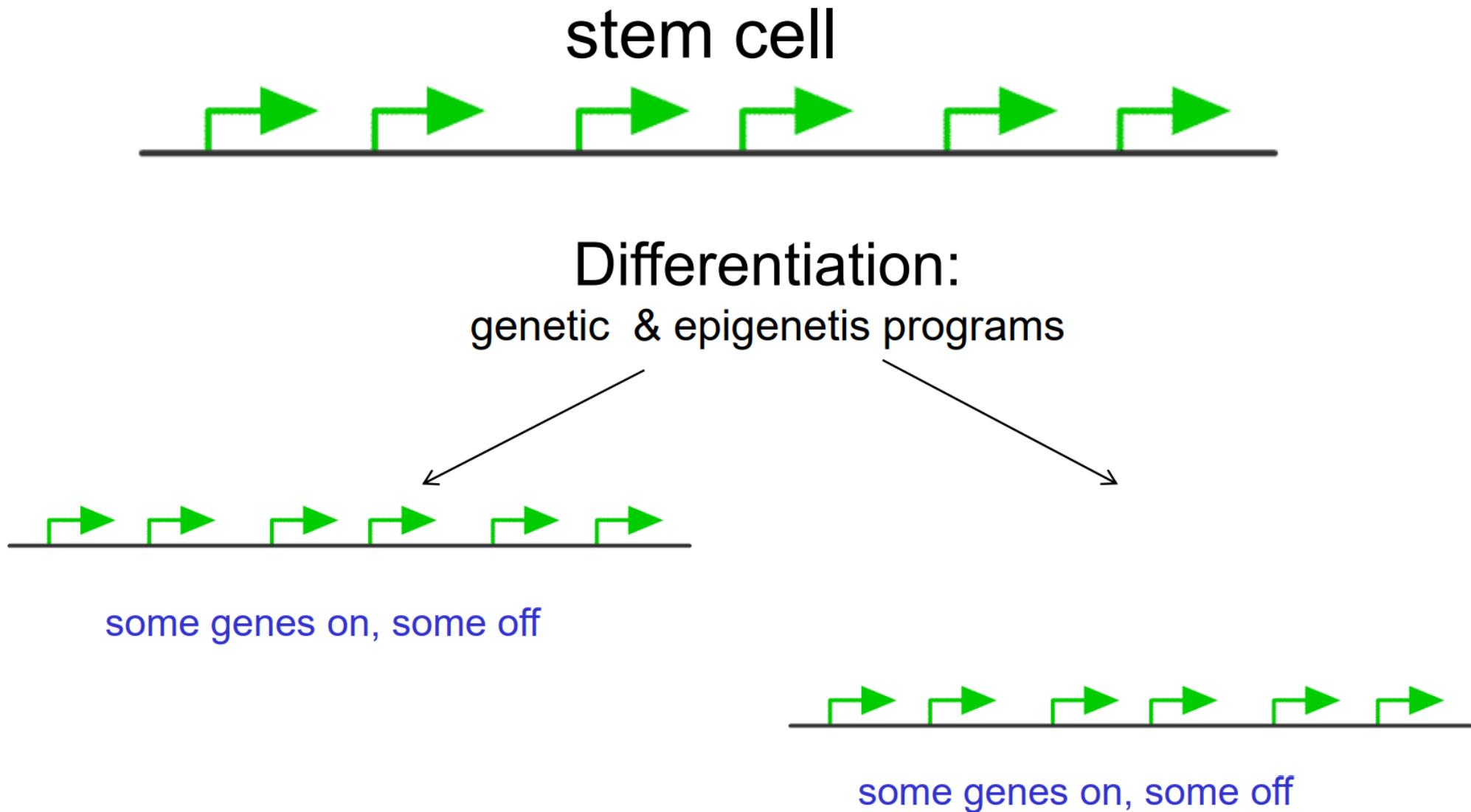


Epigenetic modifications are set and erased by specific enzymes with antagonistic specificity

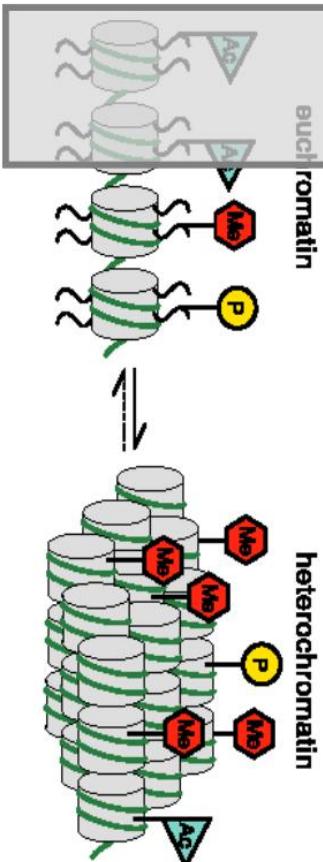


DNA-methylation & Histone modifications
are read by specific proteins

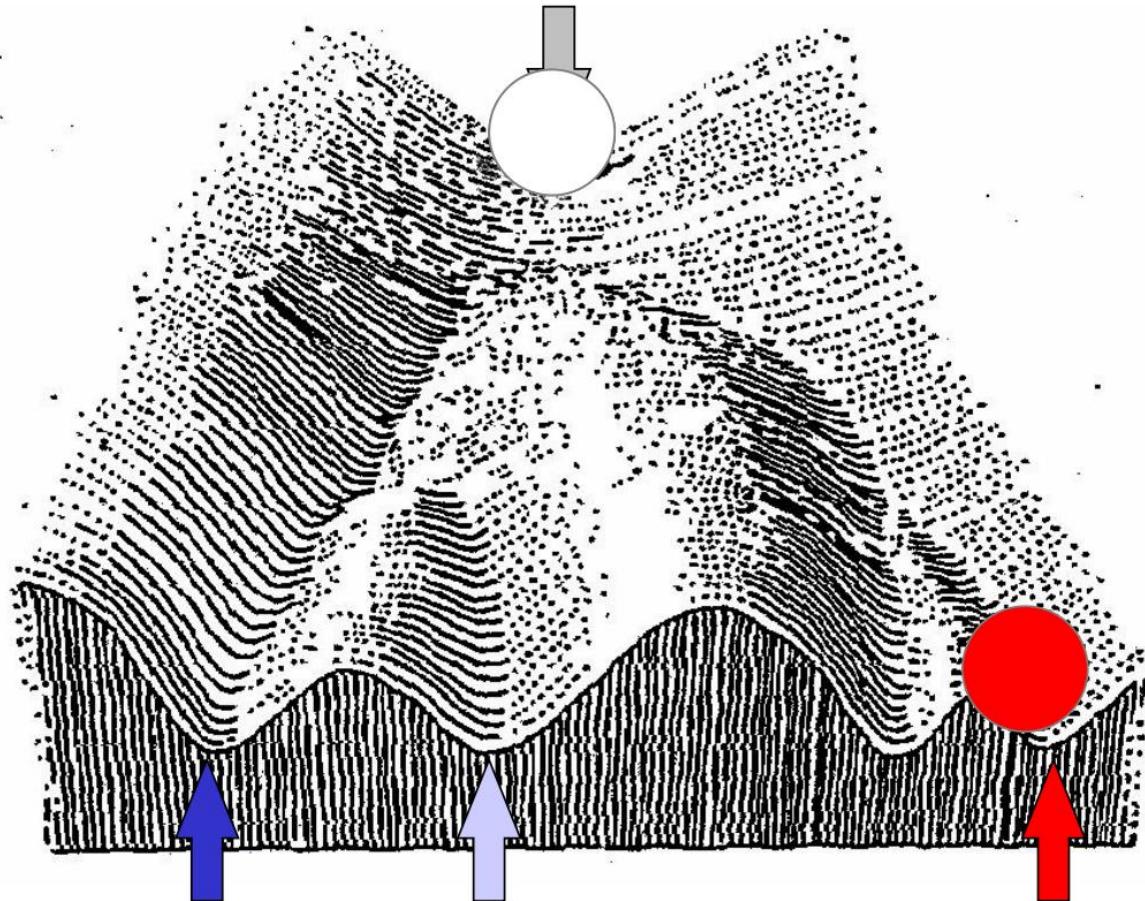
Epigenetics and development: Regulation of cell specific programs



Epigenetics and „Epigenesis“



Pluripotency



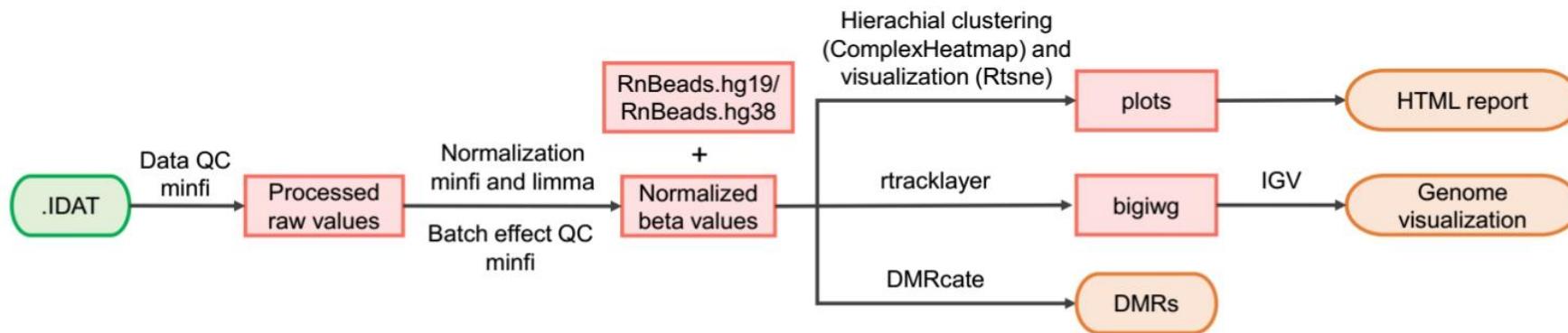
Following differentiation cells adopt different epigenomic states – reprogramming allows to revert this process

Conrad Waddington: Epigenetic landscape & "Canalization"
Modified: Timo C. Dinger & Albrecht Müller

Clinical translation I

Pharmaco-epigenomics with DAC (Decitabine) in breast cancer

DNA Methylation Array (450K and EPIC/850K) pipeline

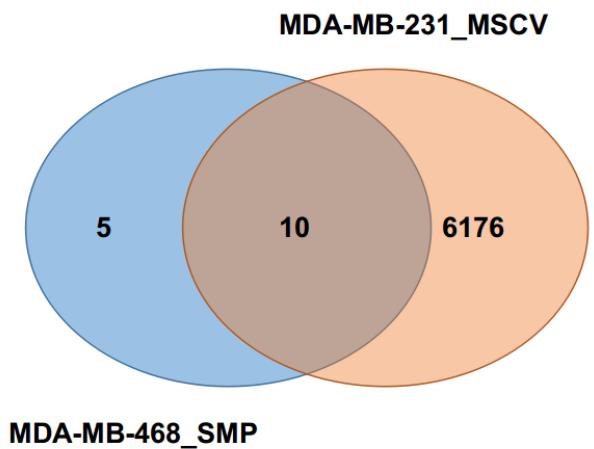
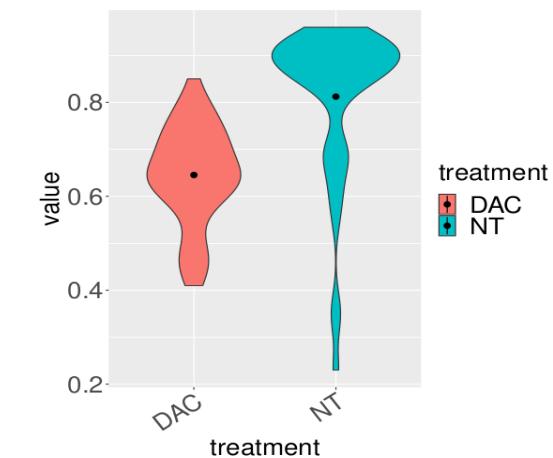
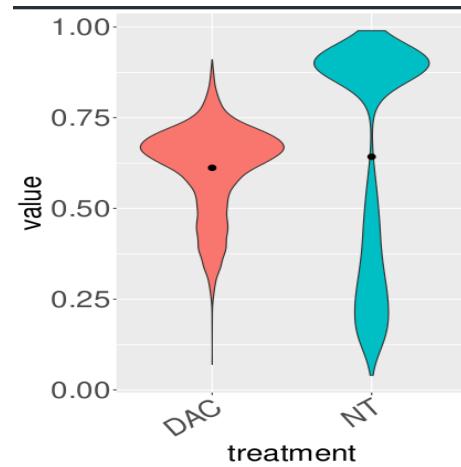


Package	Version	Link
minfi	1.34.0	http://bioconductor.org/packages/release/bioc/html/minfi.html
limma	3.44.3	https://bioconductor.org/packages/release/bioc/html/limma.html
RnBeads.hg19	1.20.0	https://bioconductor.org/packages/release/data/experiment/html/RnBeads.hg19.html
RnBeads.hg38	1.20.0	https://bioconductor.org/packages/release/data/experiment/html/RnBeads.hg38.html
Complex-Heatmap	2.4.3	https://www.bioconductor.org/packages/release/bioc/html/ComplexHeatmap.html
Rtsne	0.15	https://cran.r-project.org/web/packages/Rtsne/index.html
rtracklayer	1.48.0	https://bioconductor.org/packages/release/bioc/html/rtracklayer.html
DMRcate	2.2.3	https://bioconductor.org/packages/release/bioc/html/DMRcate.html

Figure 5. The DNA methylation array (Illumina Methylation 450K and 850K) pipeline and software.

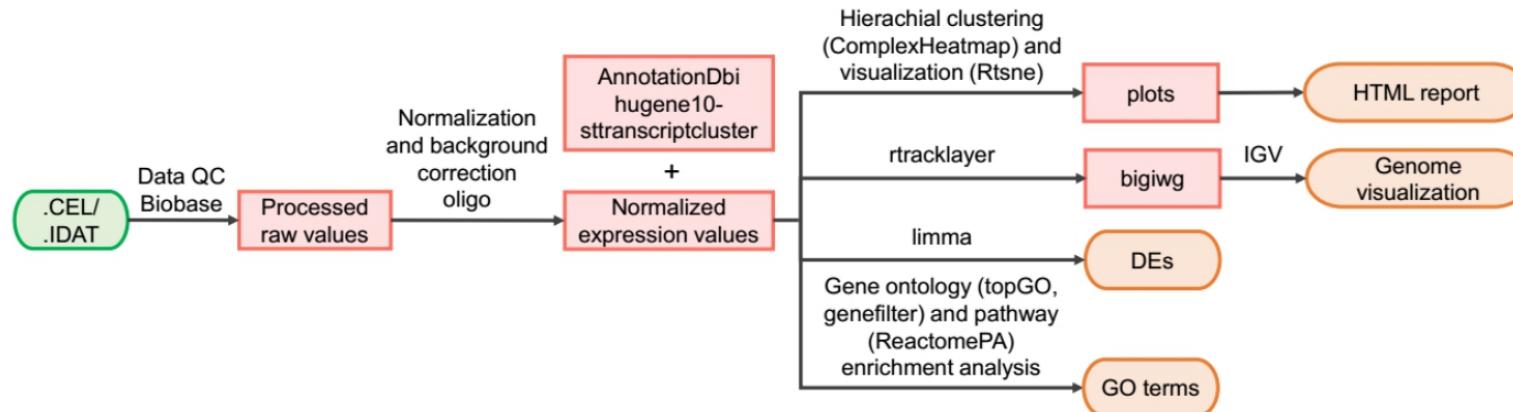
Hypomethylation in DAC group

treatment	cell.line	no.sample
DAC	MDA-MB-231_1a3	0
NT	MDA-MB-231_1a3	3
DAC	MDA-MB-231_MSCV	3
NT	MDA-MB-231_MSCV	3
DAC	MDA-MB-468_1a3	0
NT	MDA-MB-468_1a3	3
DAC	MDA-MB-468_SMP	3
NT	MDA-MB-468_SMP	3



Manuscript in preparation

Gene Expression Microarray (Affymetrix) pipeline

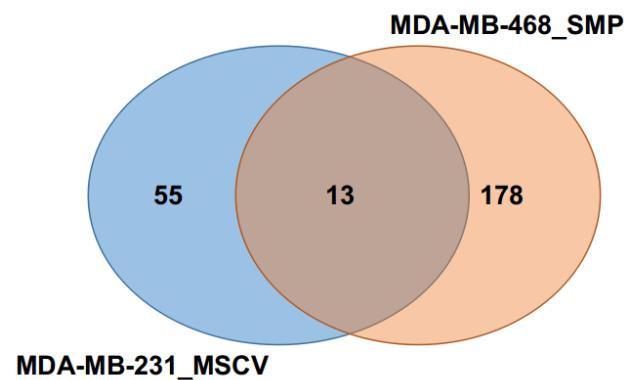
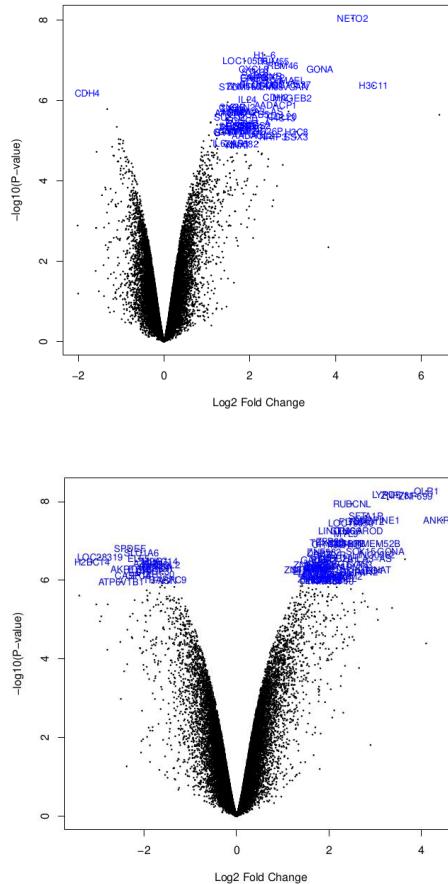


Package	Version	Link
Biobase	2.48.0	https://www.bioconductor.org/packages/release/bioc/html/Biobase.html
oligo	1.52.1	https://www.bioconductor.org/packages/release/bioc/html/oligo.html
AnnotationDbi	1.50.3	https://www.bioconductor.org/packages/release/bioc/html/AnnotationDbi.html
hugene10sttranscriptcluster	8.7.0	https://bioconductor.org/packages/release/data/annotation/html/hugene10sttranscriptcluster.db.html
Complex-Heatmap	2.4.3	https://www.bioconductor.org/packages/release/bioc/html/ComplexHeatmap.html
Rtsne	0.15	https://cran.r-project.org/web/packages/Rtsne/index.html
rtracklayer	1.48.0	https://bioconductor.org/packages/release/bioc/html/rtracklayer.html
limma	3.44.3	https://bioconductor.org/packages/release/bioc/html/limma.html
topGO	2.40.0	https://bioconductor.org/packages/release/bioc/html/topGO.html
genefilter	1.70.0	https://bioconductor.org/packages/release/bioc/html/genefilter.html
ReactomePA	1.32.0	http://bioconductor.org/packages/release/bioc/html/ReactomePA.html

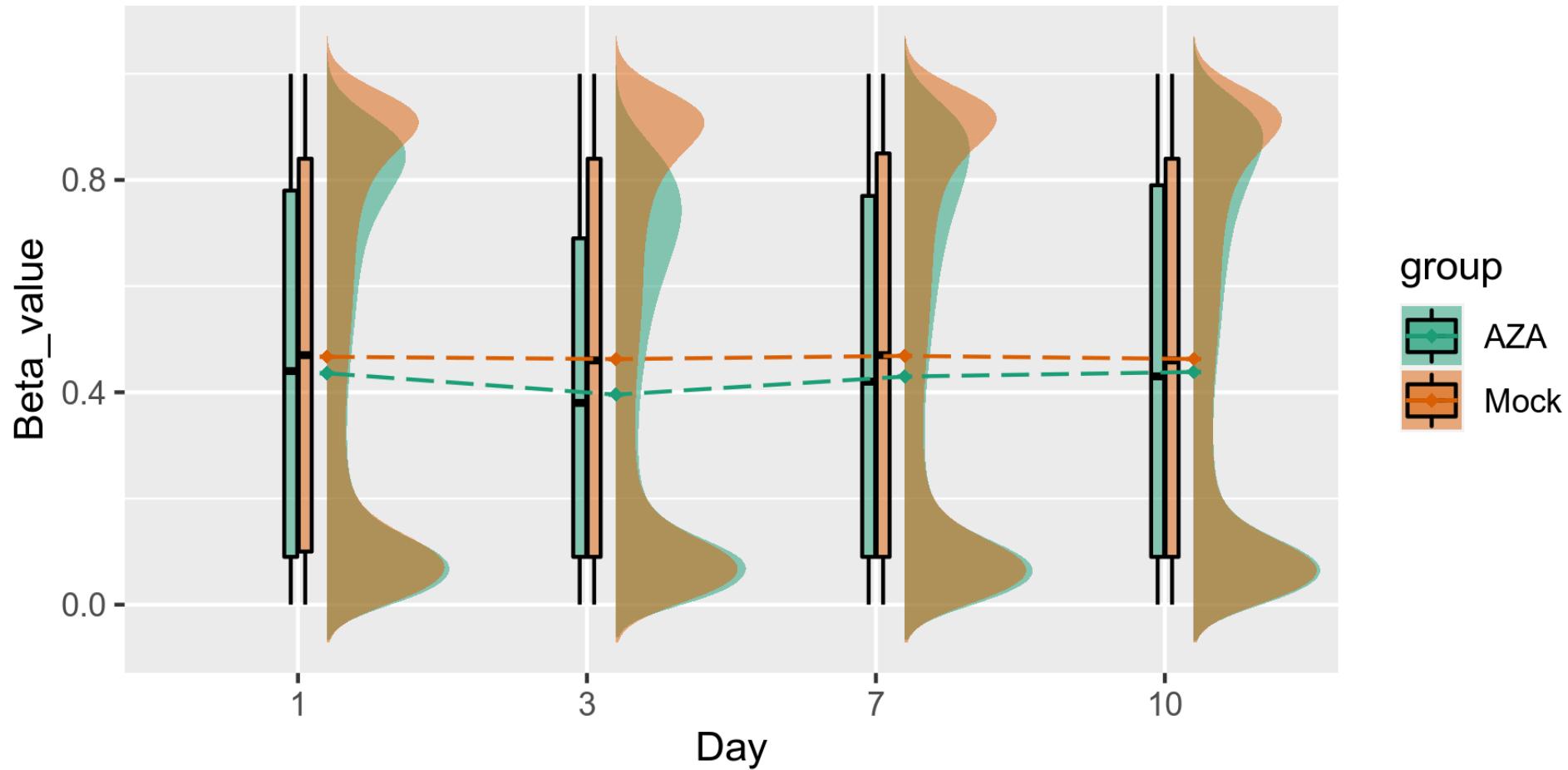
Figure 7. The Expression array (Affymetrix) pipeline and software.

Altering in DNA methylation changes gene expression

treatment	cell.line	no.sample
DAC	MDA-MB-231_1a3	0
NT	MDA-MB-231_1a3	3
DAC	MDA-MB-231_MSCV	3
NT	MDA-MB-231_MSCV	3
DAC	MDA-MB-468_1a3	0
NT	MDA-MB-468_1a3	3
DAC	MDA-MB-468_SMP	3
NT	MDA-MB-468_SMP	3



Similar observation in treatment 5 AZA in breast cancer



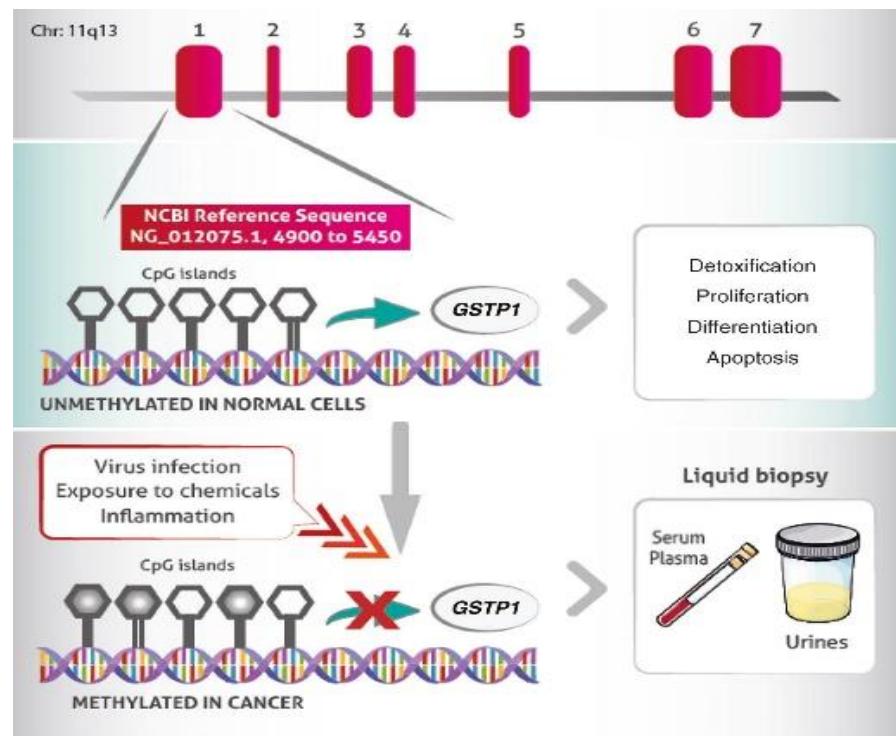
Clinical translation II, 1

mGSTP1 headloop PCR assay for monitoring for treatment metastatic
Castration-resistant prostate cancer with docetaxel

Serum Free Methylated Glutathione S-transferase 1 DNA Levels, Survival, and Response to Docetaxel in Metastatic, Castration-resistant Prostate Cancer: Post Hoc Analyses of Data from a Phase 3 Trial

Kate L. Mahon ^{a,b,c,e,†}, Wenjia Qu ^{b,†}, Hui-Ming Lin ^{b,c}, Calan Spielman ^b, Daniel Cain ^d, Cindy Jacobs ^d, Martin R. Stockler ^{a,e,f}, Celestia S. Higano ^g, Johann S. de Bono ^h, Kim N. Chi ⁱ, Susan J. Clark ^{b,c,†}, Lisa Glen Horvath ^{a,b,c,e,†,*}

^a Chris O'Brien Lifehouse, Sydney, Australia; ^b Garvan Institute of Medical Research, Sydney, Australia; ^c University of NSW, Sydney, Australia; ^d Oncogenex Pharmaceuticals Inc., Bothell, WA, USA; ^e University of Sydney, Sydney, Australia; ^f National Health and Medical Research Council Clinical Trials Centre, Sydney, Australia; ^g University of Washington, Fred Hutchinson Cancer Research Centre, Seattle, WA, USA; ^h Royal Marsden Hospital and Institute of Cancer Research, London, UK; ⁱ University of British Columbia, BC Cancer Agency, Vancouver Prostate Centre, Vancouver, BC, Canada



[10.1515/cclm-2017-0703](https://doi.org/10.1515/cclm-2017-0703)

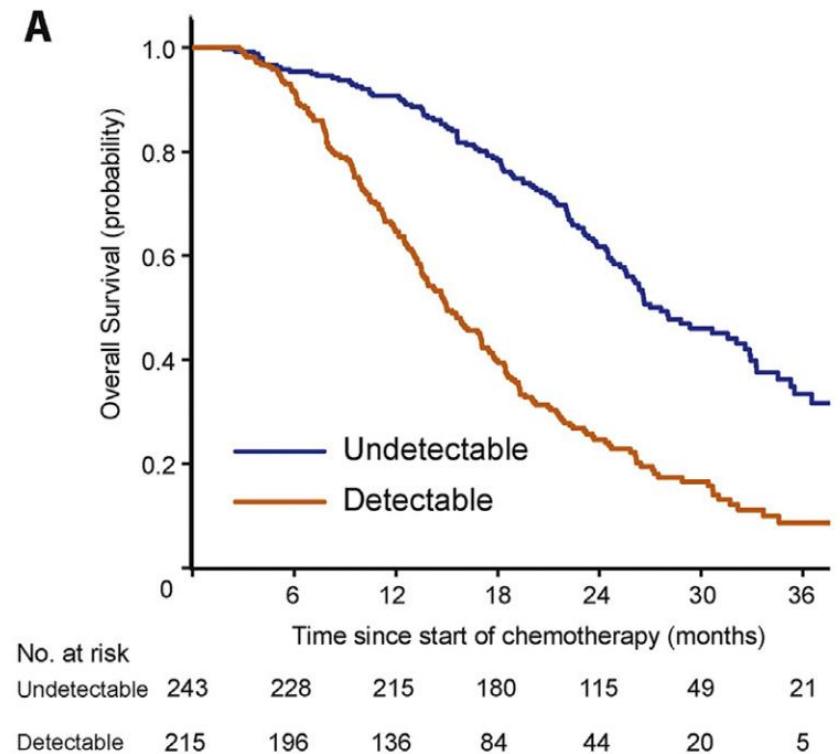


Fig. 3 – Kaplan-Meier estimates of survival in patients with a detectable mGSTP1 at baseline. (A) OS according to mGSTP1 detection after two cycles of docetaxel.

Metastatic Castration-resistant prostate cancer monitoring for treatment with docetaxel using mGASTP1 headloop PCR assay

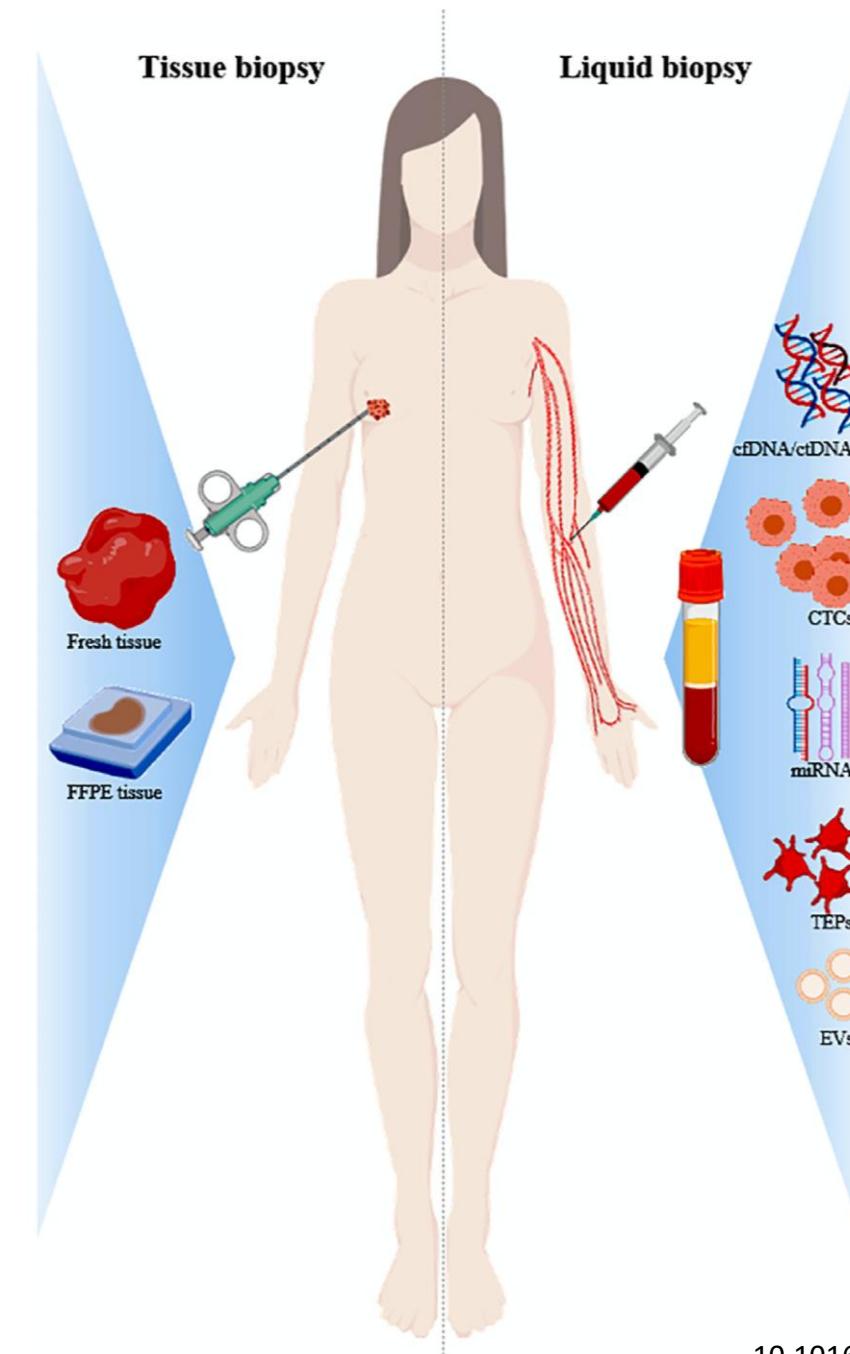
Patient ID: 13268		MF	MF	MF	MF									
Block (Pre-2014)	Block No	Specimen ID	BS Plate	Tube No	Total DNA (ng)	GSTPiHL CT1	GSTPiHL CT2	GSTPiHL CT3	Ave Total Methylated GSTPi (ng)	Control CT1	Control CT2	Control CT3	Ave Total BS DNA(ng)	
	1	1453895	A1	1	85.2	44.64	36.10	43.39	1.48	16.12	16.24	16.40	33.38	
	2	1453896	B1	2	38	43.34	36.78	43.84	1.01	17.77	17.64	17.63	12.71	
	3	1453897	C1	3	18	42.37	41.57	49.93	0.10	18.38	18.39	18.47	7.73	
	4	1453898	D1	4	10	55.13	52.74	Undetermined	#VALUE!	20.04	20.11	19.89	2.62	
	5	1453899	E1	5	46.4	51.91	49.85	49.47	0.00	17.78	17.81	17.85	11.59	
	6	1453900	F1	6	64	39.88	41.87	40.89	0.30	16.81	17.02	16.91	21.36	
	7	1453901	G1	7	37.4	56.68	49.99	49.55	0.00	17.65	17.59	17.74	12.84	
	8	1453902	H1	8	58.4	37.09	36.36	36.77	3.04	17.42	17.52	17.63	14.11	
	9	1453903	A2	9	46.4	45.17	40.05	38.89	0.44	17.31	17.26	17.40	16.17	
	10	1453904	B2	10	136	40.66	40.25	36.54	1.35	15.91	15.92	15.82	42.81	
	11	1453905	C2	11	188	36.59	36.62	36.13	3.59	16.25	16.14	16.18	34.79	
	12	1453906	D2	12	65.2	44.77	39.06	37.14	1.06	17.21	17.14	17.12	18.04	
	13	1453907	E2	13	26.2	49.91	45.66	49.04	0.01	17.93	17.91	18.00	10.58	
	14	1453908	F2	14	28.2	50.18	49.83	46.54	0.00	17.73	17.76	17.97	11.54	
	15	1453909	G2	15	59.6	50.64	46.70	38.32	0.40	16.98	17.09	17.27	18.63	
	16	1453910	H2	16	52.8	37.82	49.00	49.32	0.53	17.81	17.94	17.92	11.01	
	17	1453911	A3	17	18.2	Undetermined	52.76	59.25	#VALUE!	18.86	18.76	18.85	5.85	
	18	1453912	B3	18	2.8	51.65	53.99	Undetermined	#VALUE!	21.35	21.35	21.40	1.04	
Block (Post-2019)	1	1453914	C3	21	354	36.33	35.58	34.63	6.65	13.20	13.37	13.36	244.49	
	2	1453915	D3	22	74.4	45.29	47.05	45.84	0.01	14.00	14.03	13.98	152.53	
	3	1453916	E3	23	182.4	35.70	36.23	35.07	5.81	12.81	12.98	12.93	321.46	
	4A	1453917	F3	24	440	35.91	36.65	35.85	4.35	13.01	13.09	13.02	293.19	
	4B	1453928	G3	25	146.8	37.93	35.81	37.74	2.77	12.93	13.01	13.11	298.63	
	5	1453918	H3	26	131.6	39.14	42.97	40.77	0.37	13.17	13.02	13.15	278.59	
	6	1453919	A4	27	82.4	37.63	40.19	40.64	0.83	13.40	13.38	13.46	227.71	
	7	1453920	B4	28	61.2	46.81	45.75	46.99	0.01	13.97	14.13	14.16	144.63	
	8	1453921	C4	29	9.2	49.48	47.88	48.59	0.00	15.91	15.86	15.92	42.32	
	9	1453922	D4	30	35.6	44.96	38.49	47.37	0.37	15.19	15.16	15.23	68.22	
	10	1453923	E4	31	191.6	37.30	36.28	36.91	2.93	13.01	13.06	13.11	289.79	
	11	1453924	F4	32	424	34.52	33.58	34.50	13.65	13.46	13.49	13.52	216.46	
	12	1453925	G4	33	133.2	32.83	33.40	32.68	27.40	13.13	13.13	13.29	266.47	

Clinical translation II, 2

Liquid biopsy (cfDNA) in breast cancer diagnosis

Aims of the study

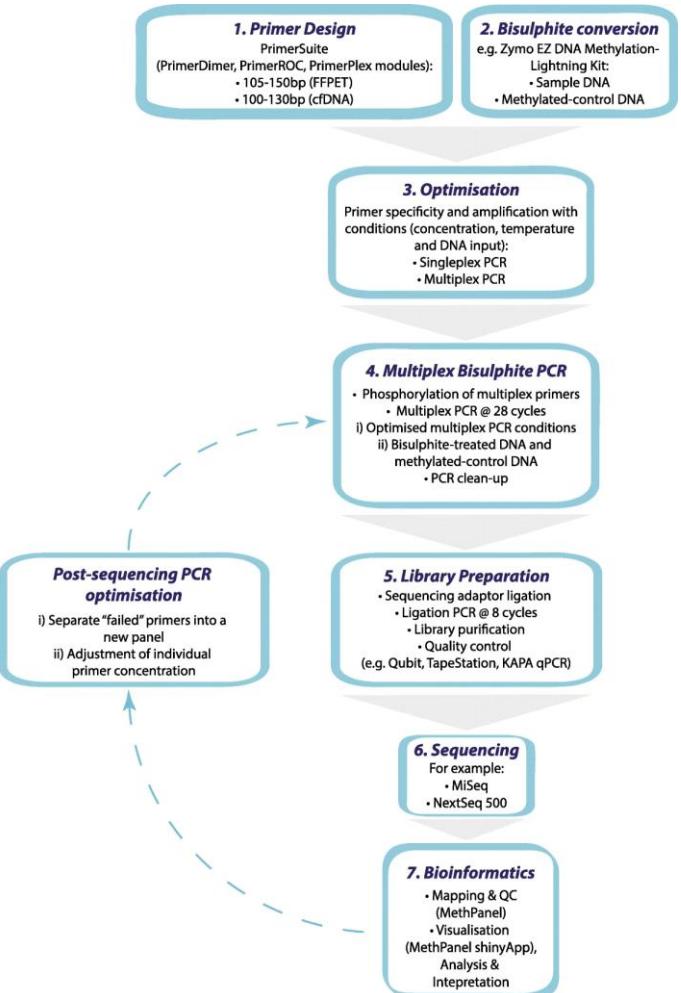
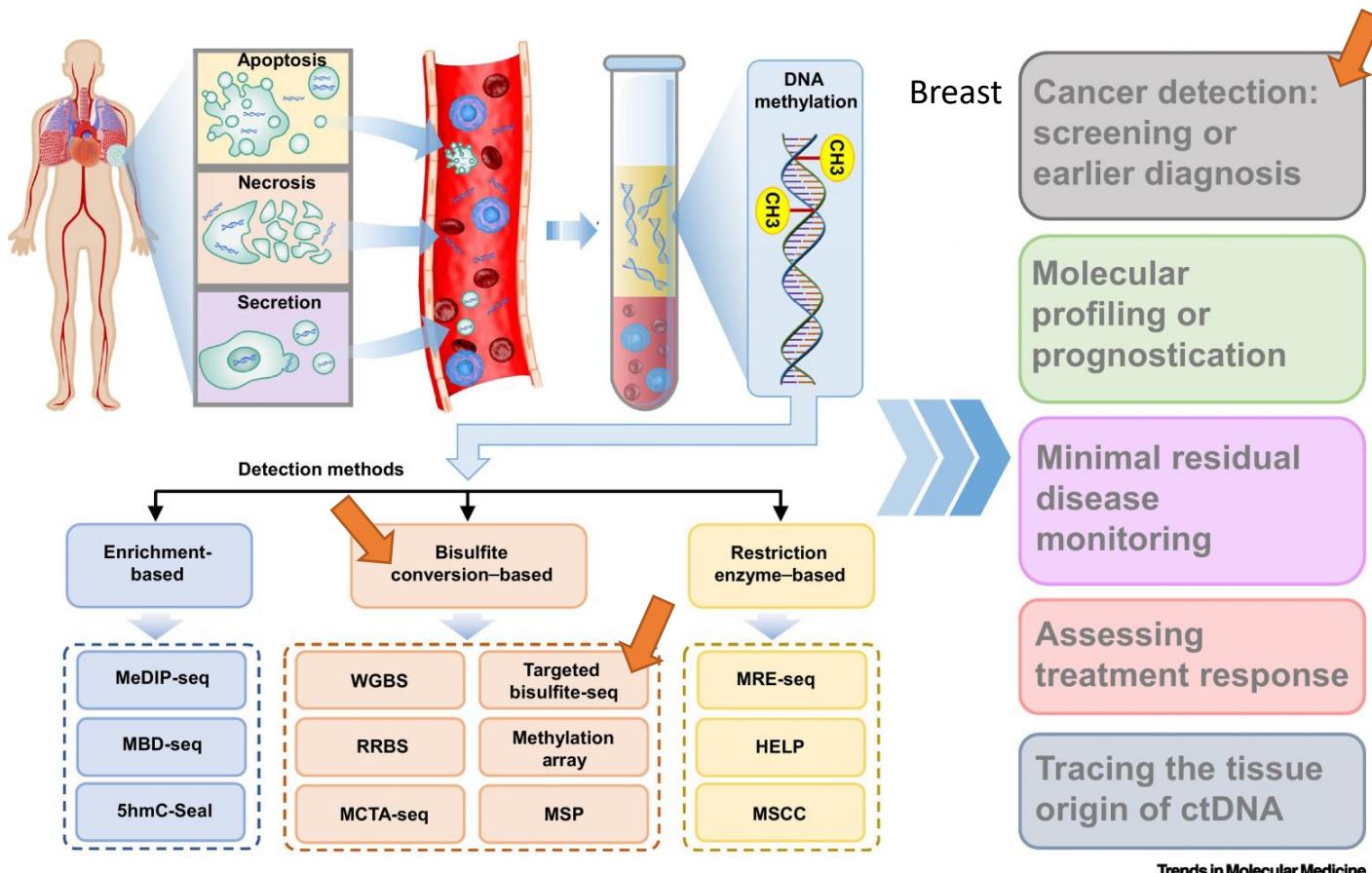
- Build up DNA methylation panel for early detection of breast cancer
- Validate the panel
 - Tissue biopsy of patients and healthy controls (**Tissue/FFPE**)
 - Liquid biopsy of patients and healthy controls (**cfDNA**)



Machine learning techniques

- Gmlnet, PLS and MVUR for feature selection
- Random Forest for classification
- Smote for up sampling in unbalance dataset of cfDNA

Targeted multiplex bisulphite PCR sequencing

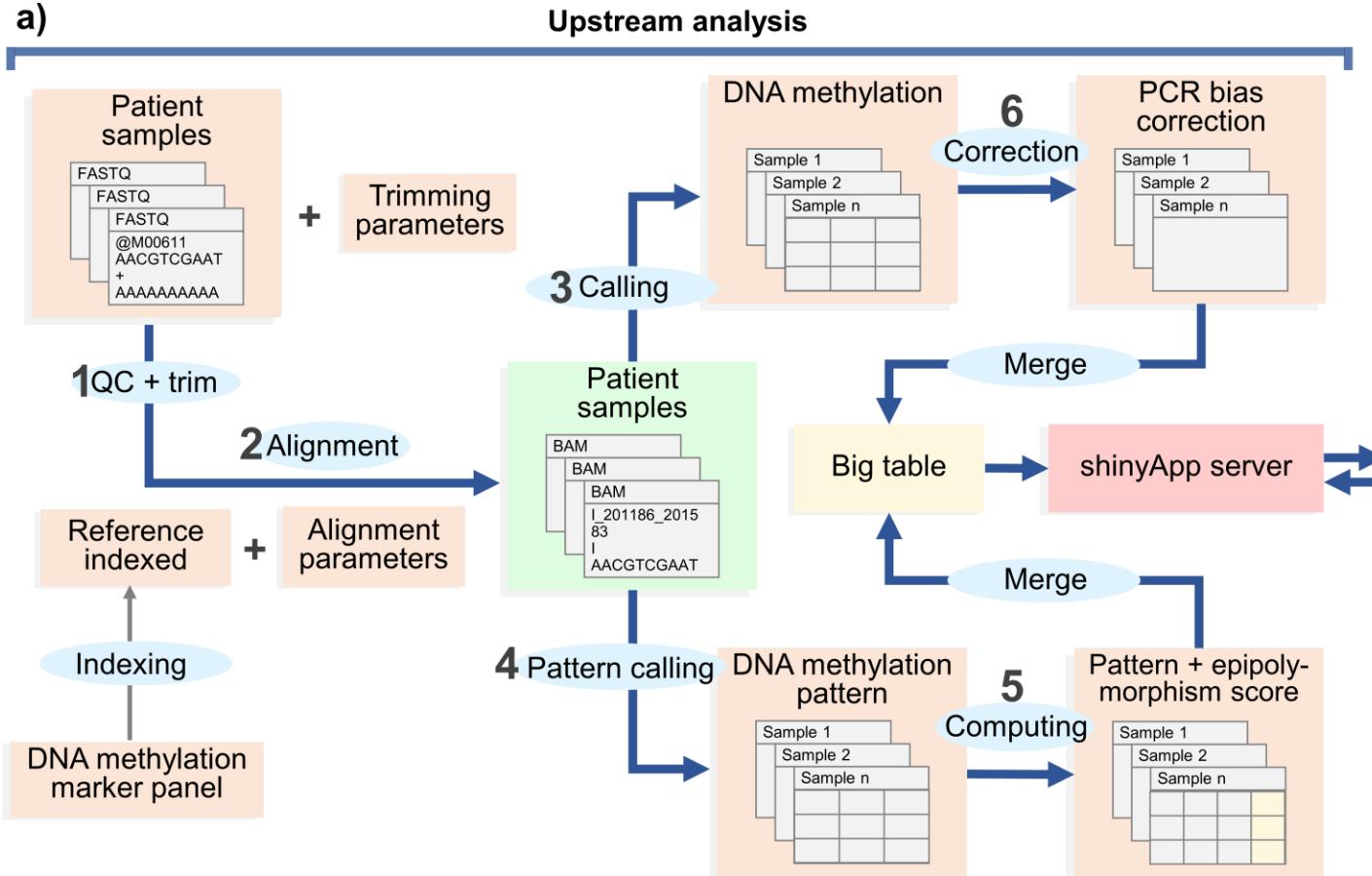


Lam D*, Luu PL*, Song JZ, Qu W, Risbridger GP, Lawrence MG... SJ Clark, R Pidsley, Stirzaker C. Comprehensive evaluation of targeted multiplex bisulphite PCR sequencing for validation of DNA methylation biomarker panels. Clin Epigenetics. 2020;12(1):90. * Equal contribution authors

Luo H., Wei W., Ye Z., Zheng J., Xu R. Liquid biopsy of methylation biomarkers in cell-free DNA. Trends Mol. Med. 2021

MethPanel: analysing and visualizing amplicon bisulphite sequencing data

a)

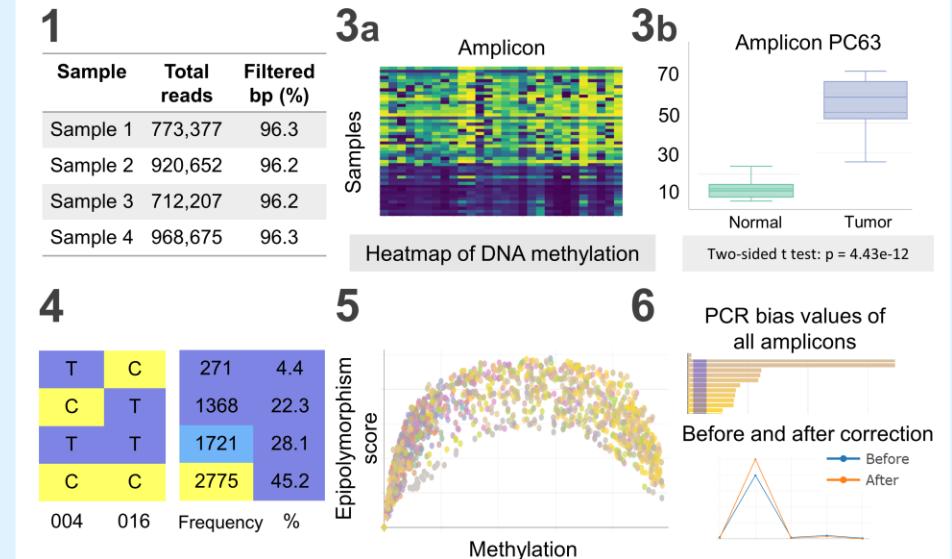


b)

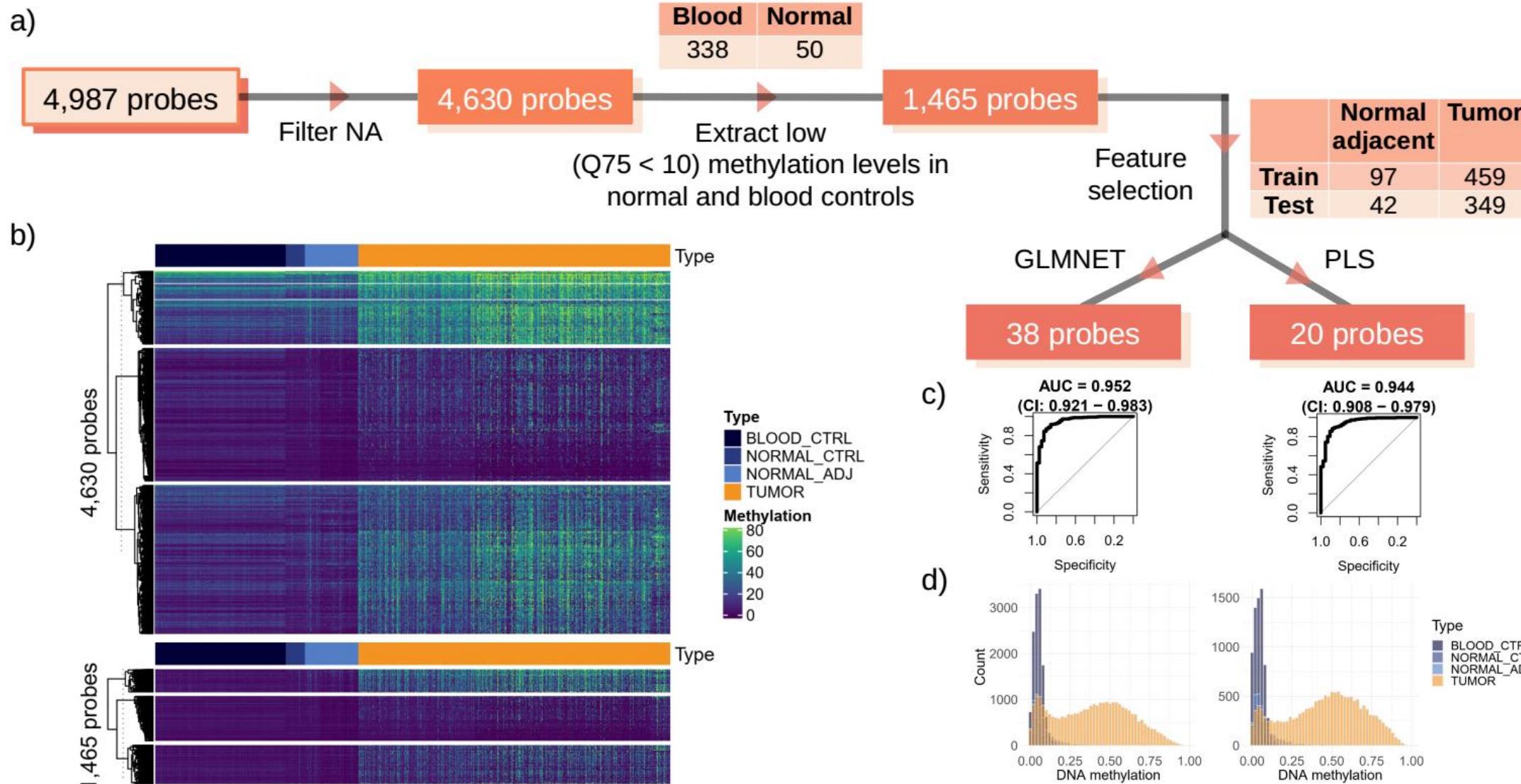
shinyApp client

MethPanel

Login 1. Trimmed FASTQ QC 2. Alignment 3. DNA methylation 4. Pattern 5. Epipolymerism 6. PCR bias correction 7. DNA methylation after correction

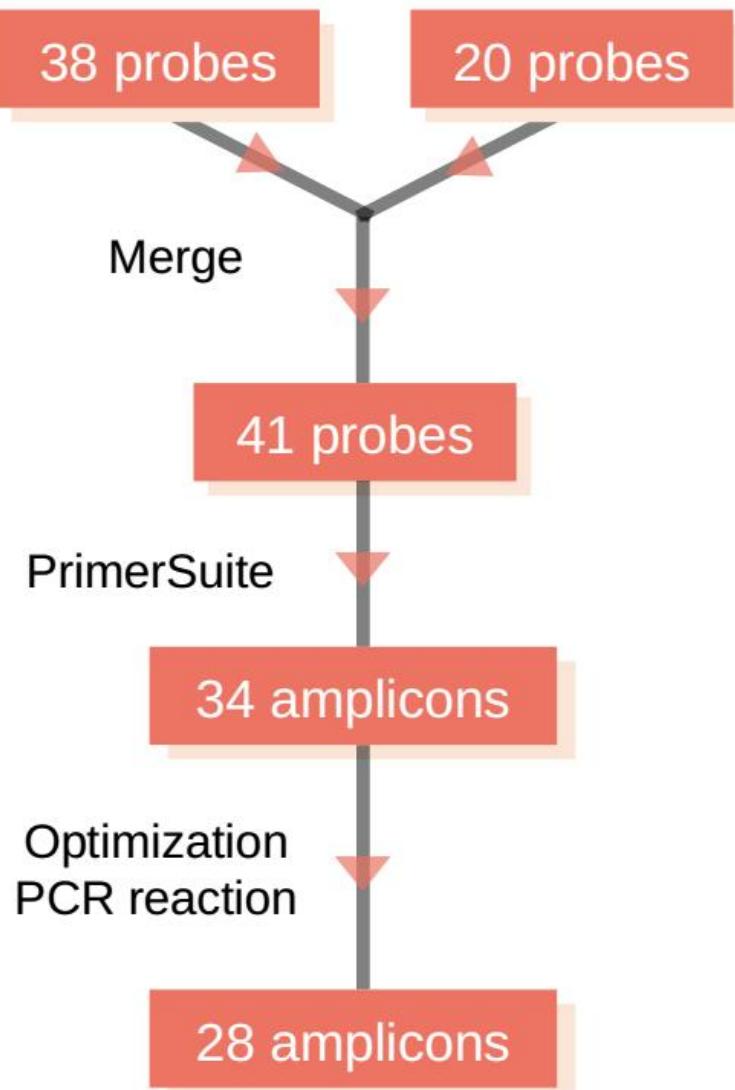


Build up DNA methylation breast cancer panel



Characterization of DNA methylation breast cancer panel

a)

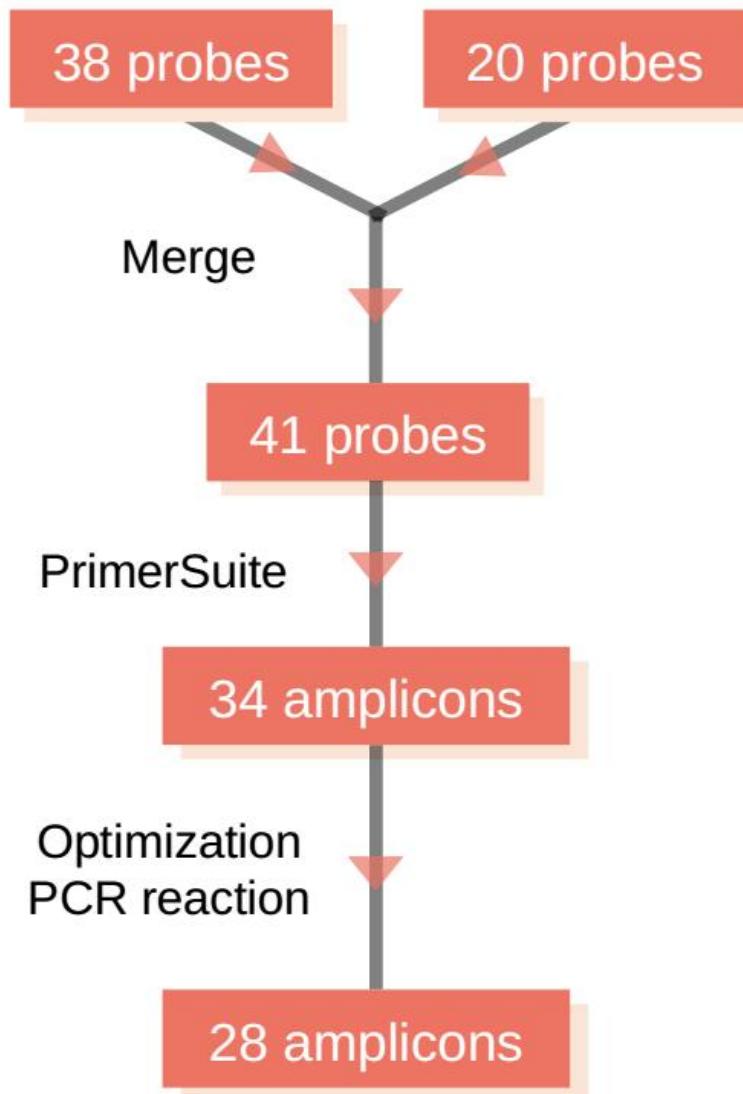


Check pseudogenes and mapping ability of these 28 amplicons:

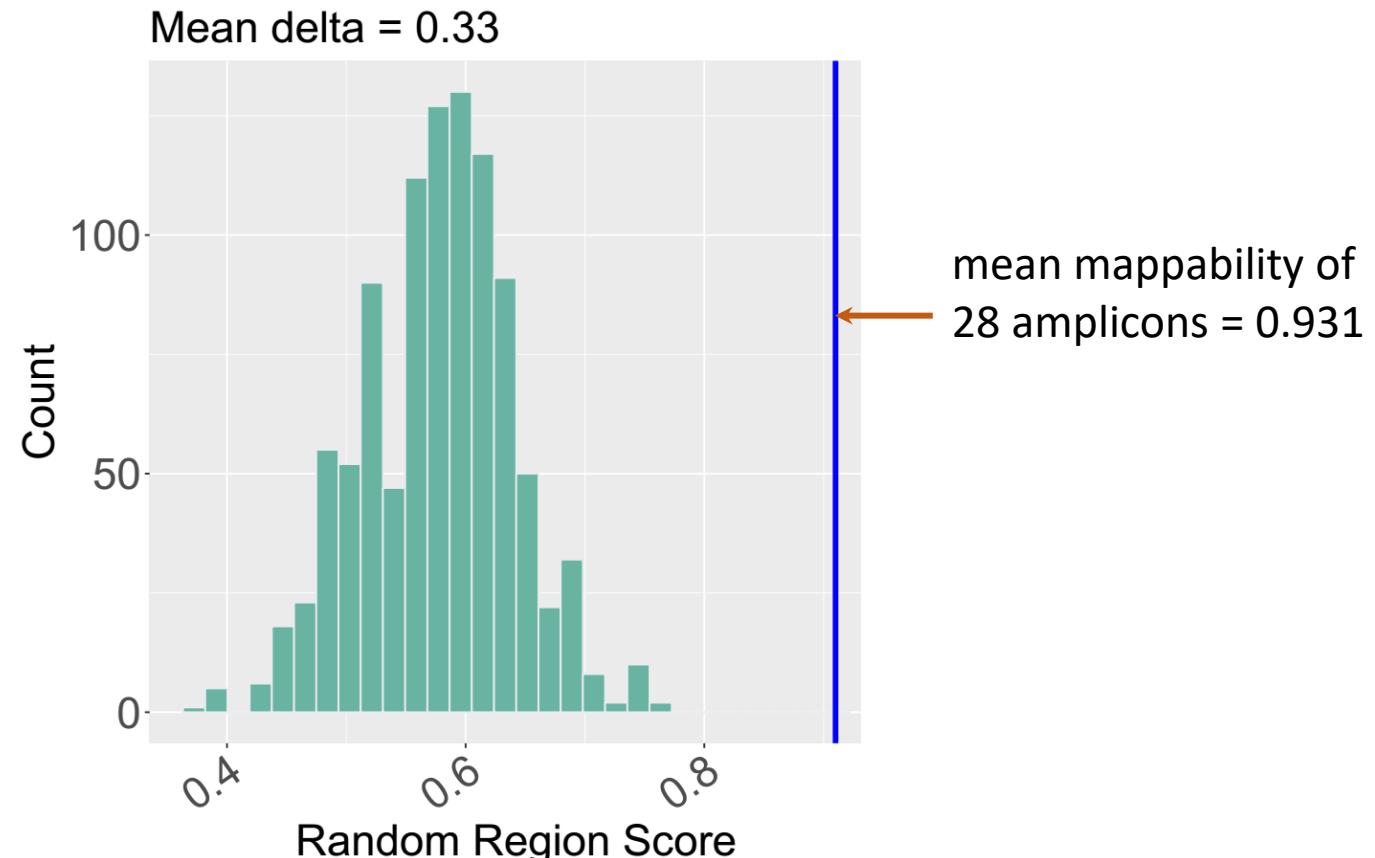
- 1) ENCODE Black list: NO
- 2) DUKE Black list: NO
- 3) Sanger Dead Zone: NO
- 4) NGS Dead Zone: NO
- 5) ENCODE Mapability
- 6) DUKE Mapability Uniqueness
- 7) NGS Problem High Mapping: PC66_UBD in chrom 6, MHC region
- 8) NGS Problem Low Mapping: NO

Characterization of DNA methylation breast cancer panel

a)



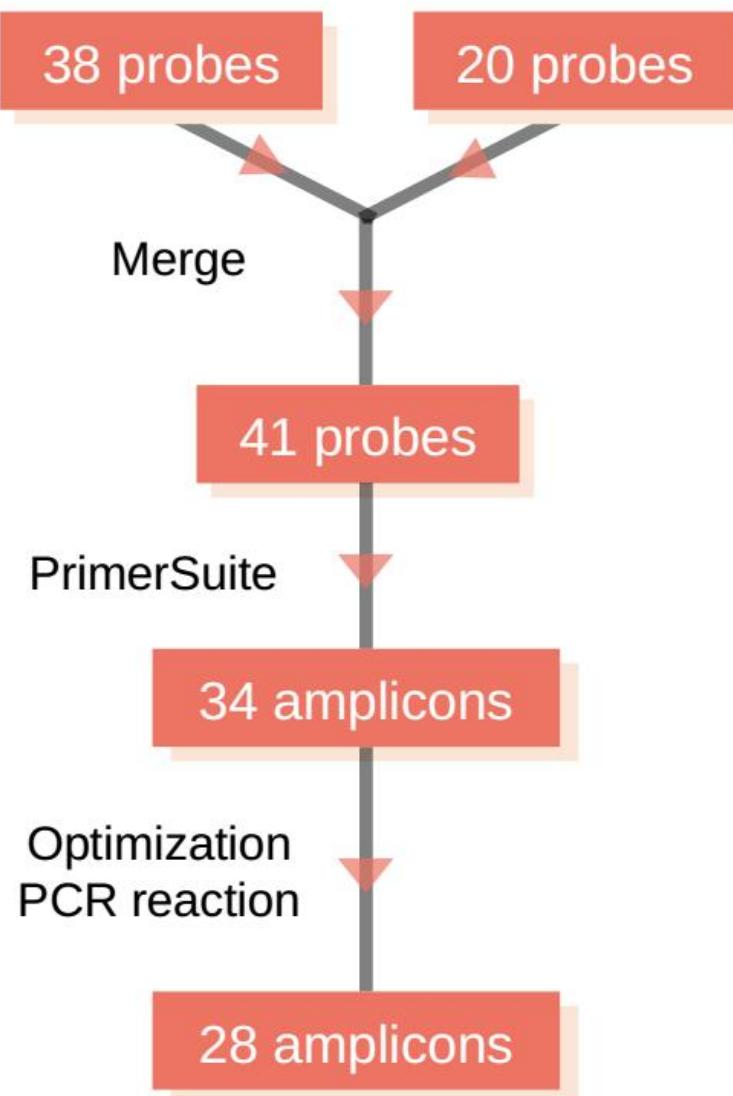
Check pseudogenes and mapping ability of these 28 amplicons:
5) ENCODE Mapability: 1000 times randomize
 $-1 \leq \text{Mapability score} \leq 1$



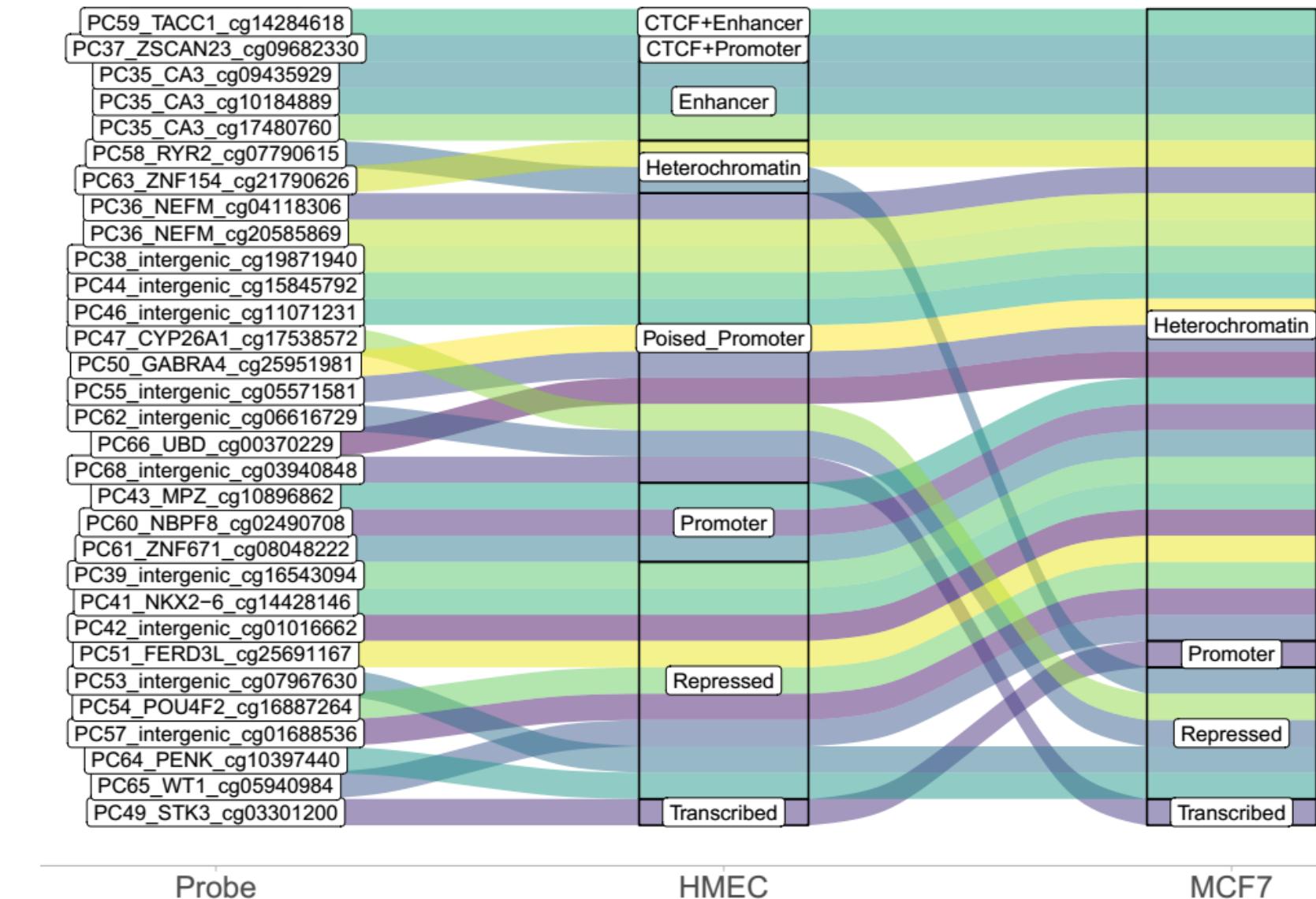
Results

Characterization of DNA methylation breast cancer panel

a)

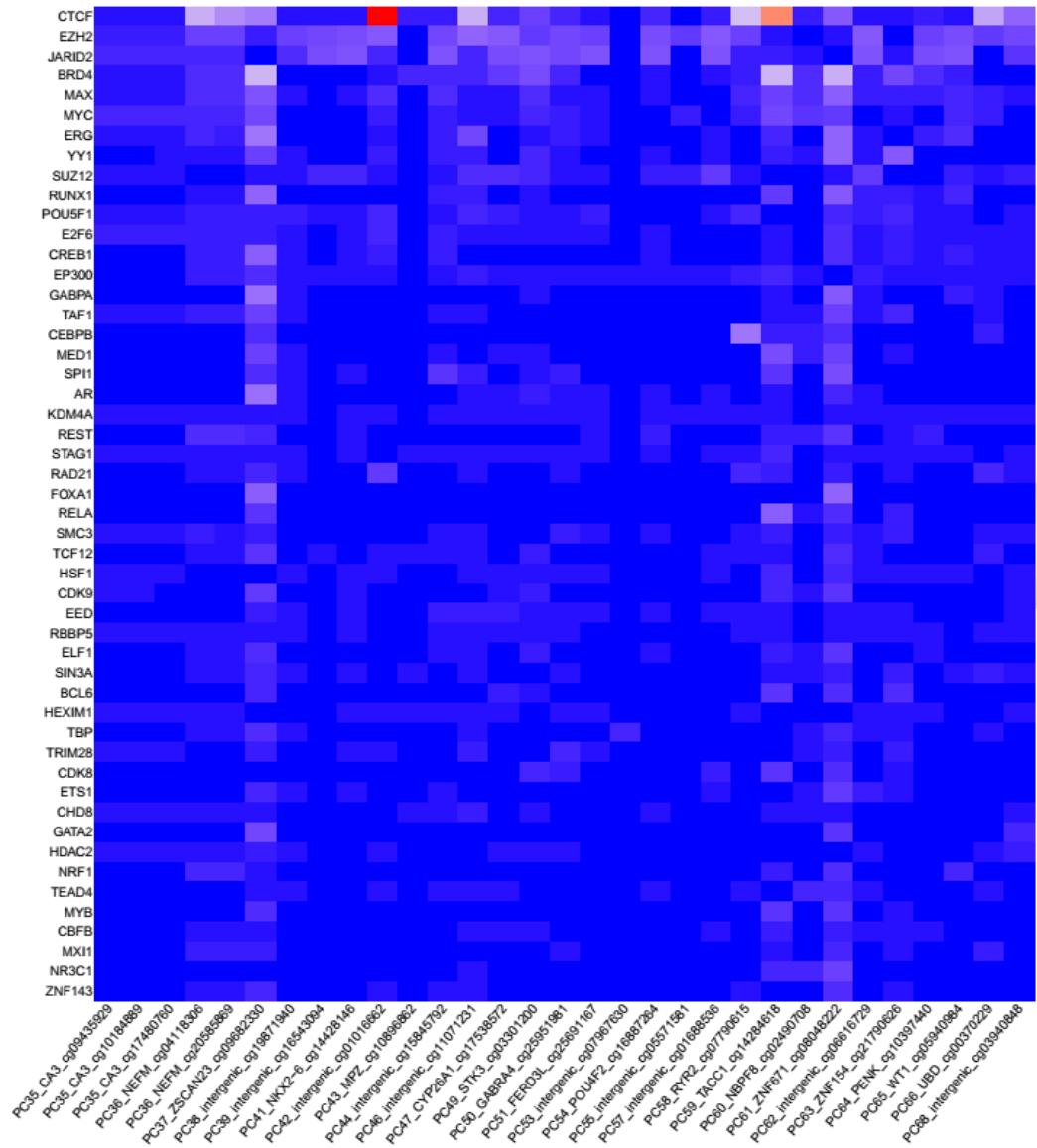


b) ChromHMM



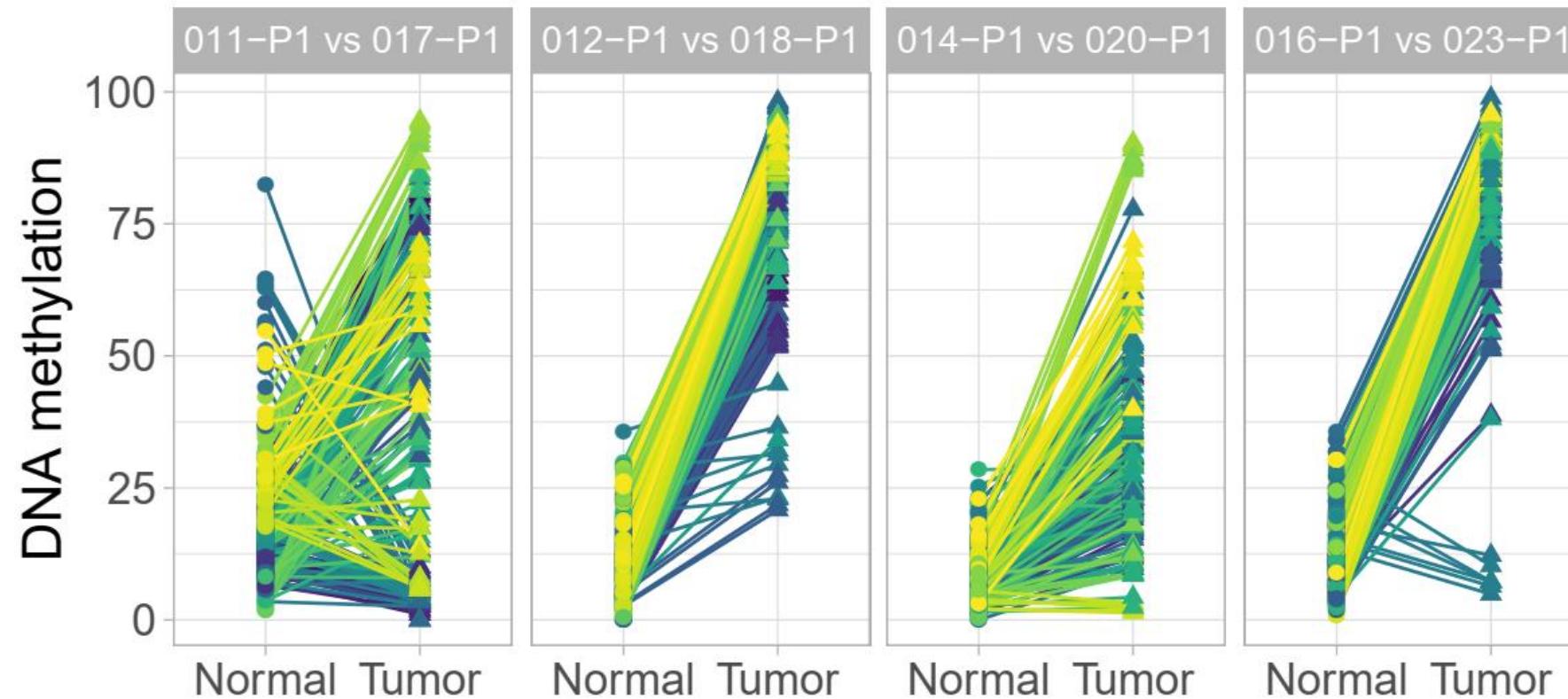
Characterization of DNA methylation breast cancer panel

c) TFBS



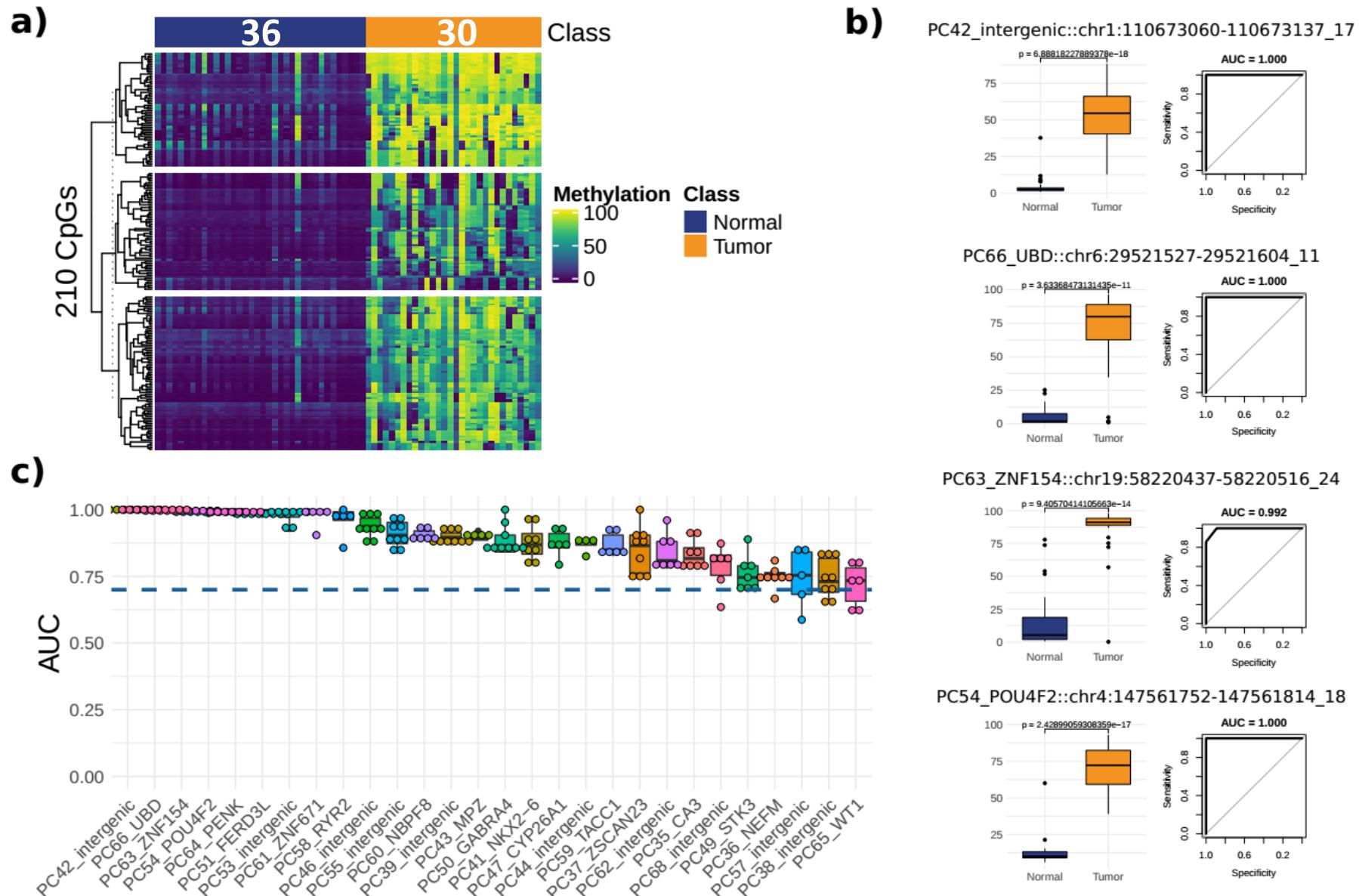
Validation of the breast cancer panel on Tissue

4 pairs of normal and tumor of the same patients



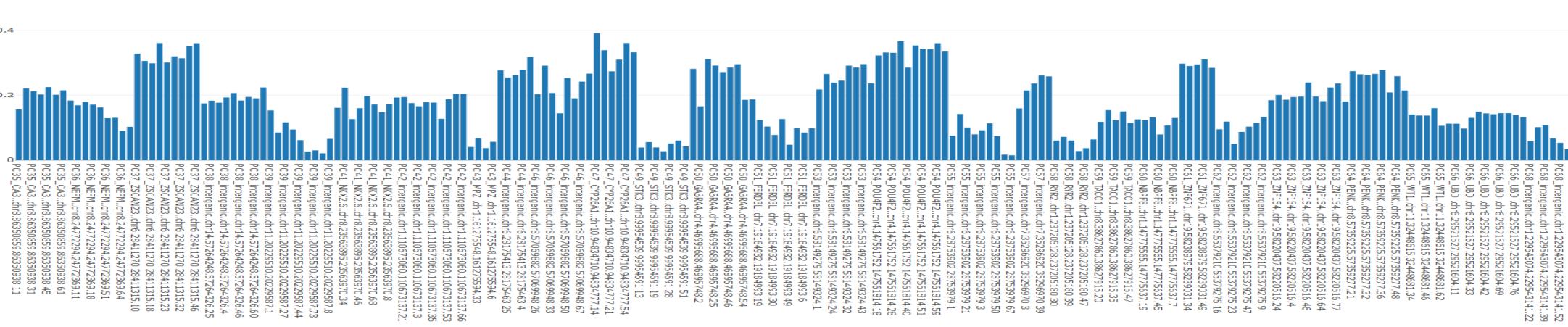
Validation of the breast cancer panel on Tissue/FFPE

Normal (36)		Tumor (30)
Blood	Normal	Tumor
20	16	30

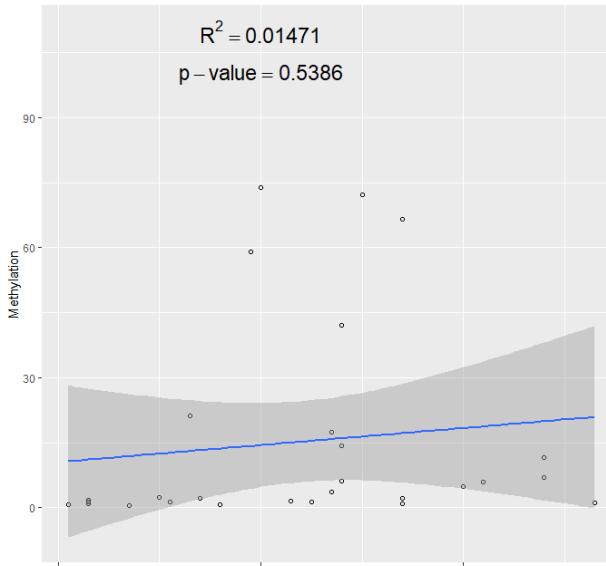


No significant correlation between DNA methylation and age (confound factor)

$R^2 = 0.5$

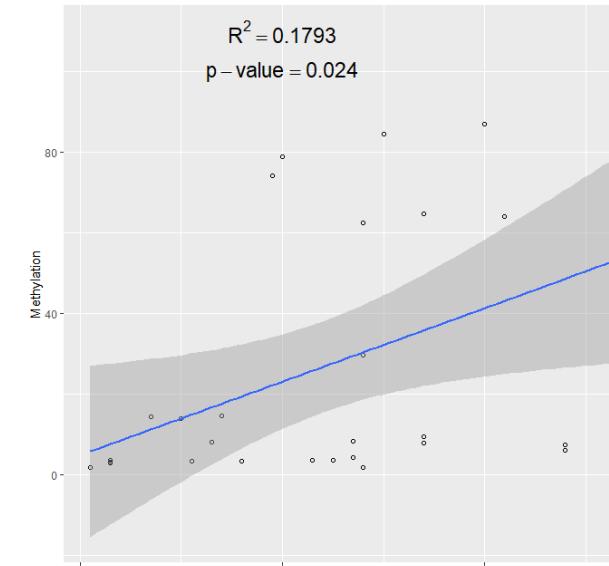


PC55_intergenic..chr6.28753902.28753979.67



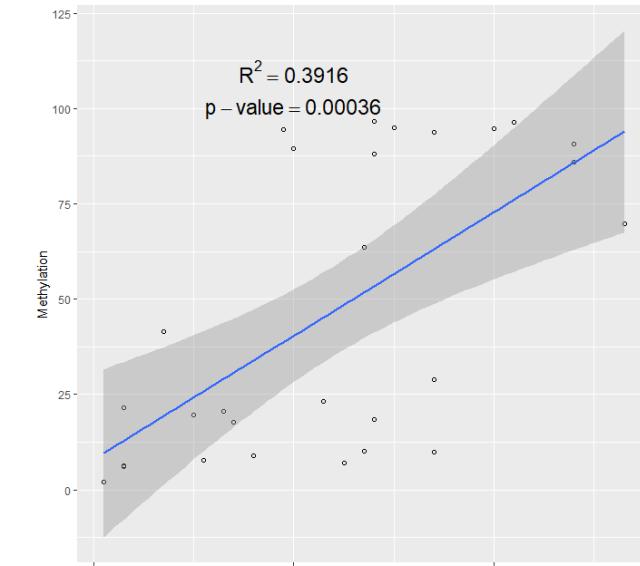
Minimum R^2

PC36_NEFM..chr8.24772294.24772369.16



Median R^2

PC47_CYP26A1..chr10.94834710.94834777.14

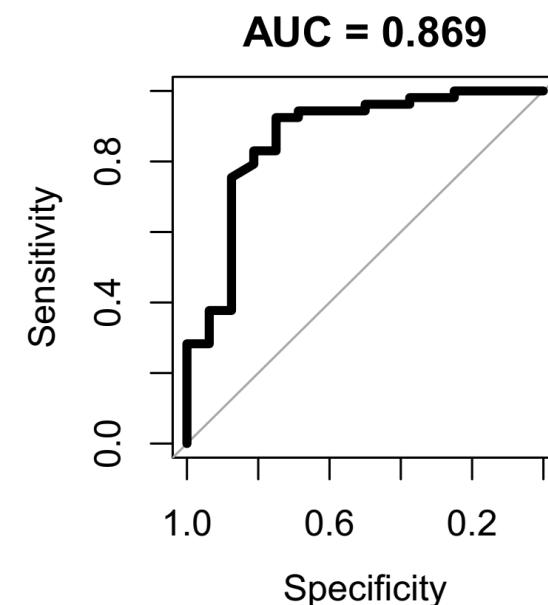
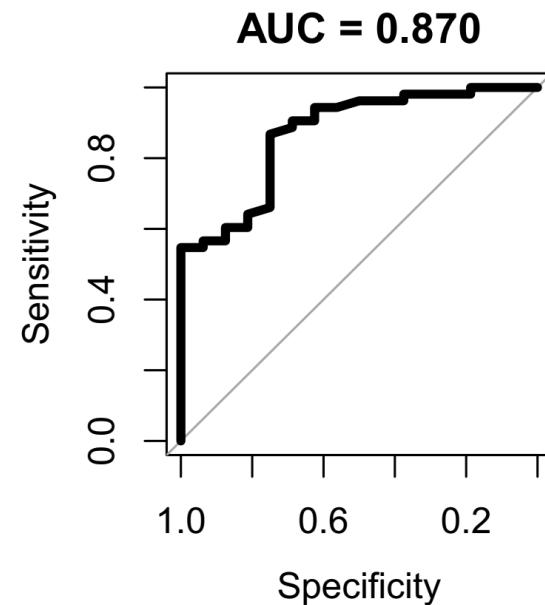


Maximum R^2

Validation of the breast cancer panel cfDNA

Liquid Biopsy (cfDNA)		
Normal (66)		Tumor (215)
Blood	Normal	Tumor
20	46	215

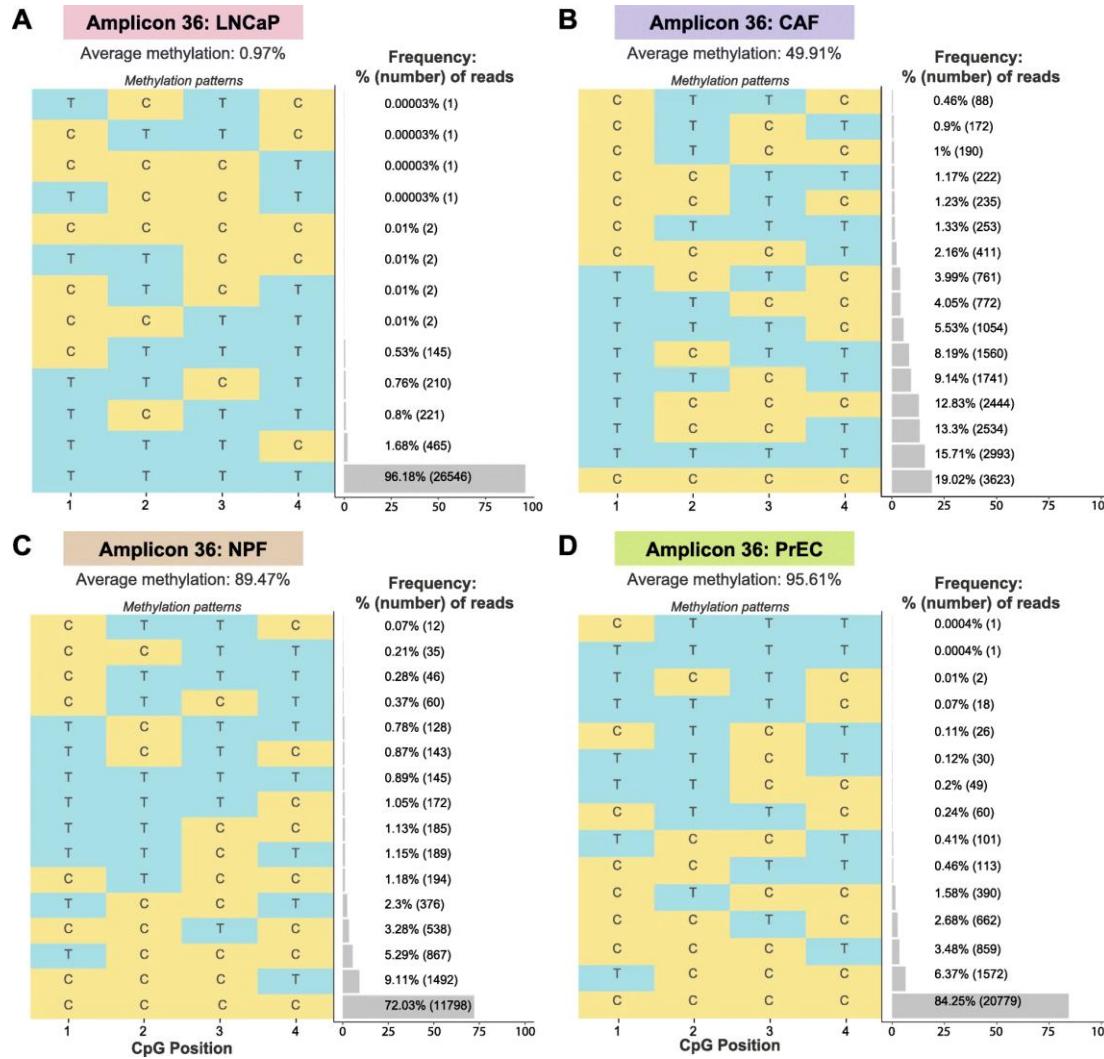
order	name	rank
1	PC63_ZNF154::chr19:58220437-58220516_24	1.891429
2	PC66_UBD::chr6:29521527-29521604_11	2.46
3	PC60_NBPF8::chr1:147775565-147775637_4	3.405
4	PC51_FERD3L::chr7:19184932-19184993_49	5.479643
5	PC59_TACC1::chr8:38627860-38627915_38	6.851071
6	PC46_intergenic::chr8:57069882-57069948_67	7.403571



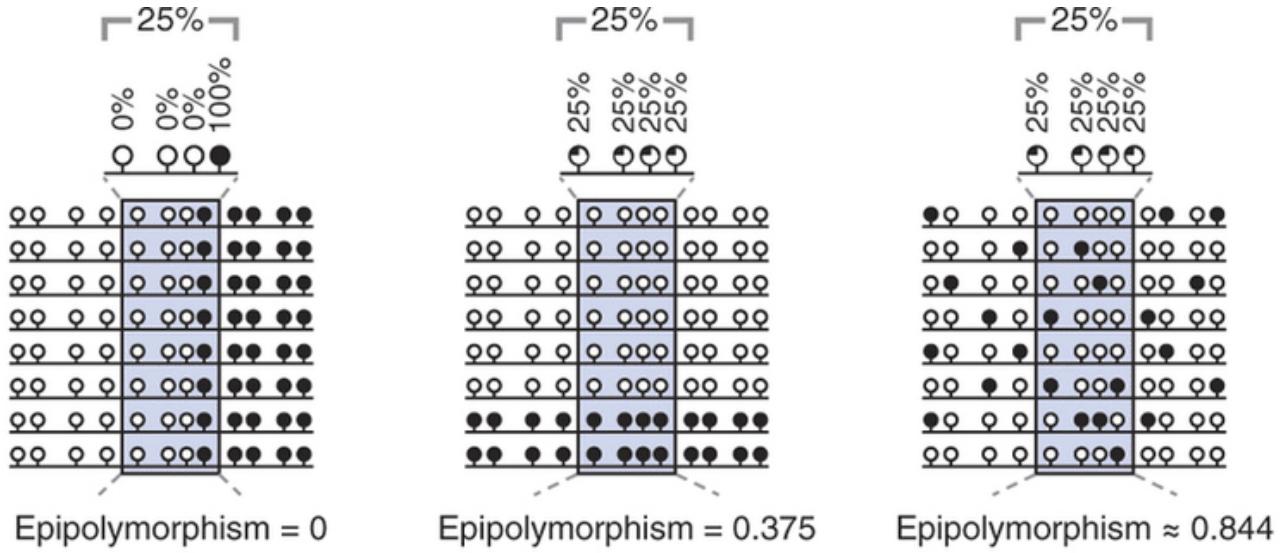
10 significant CpGs (T test, p value < 0.05)

6 important CpGs (from feature selection package MVUR)

DNA methylation pattern

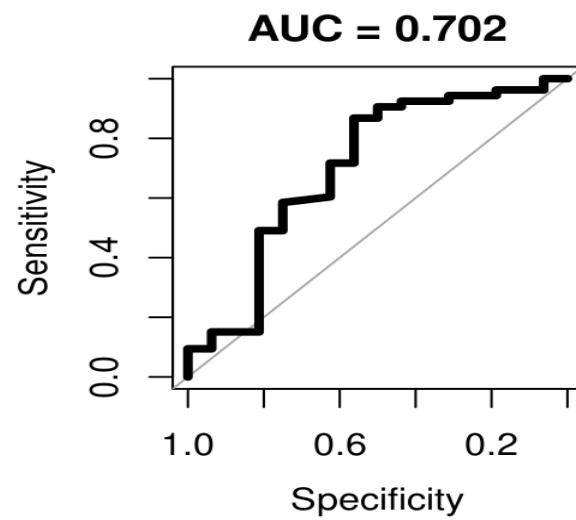


Epipolymorphism

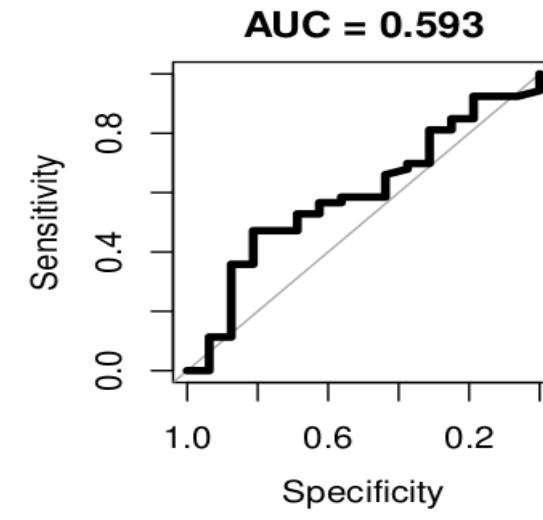


Pattern and EpiPolymorphism score on cfDNA

Liquid Biopsy (cfDNA)		
Normal (66)		Tumor (215)
Blood	Normal	Tumor
20	46	215



Pattern



EpiPolymorphism score

EpiClass

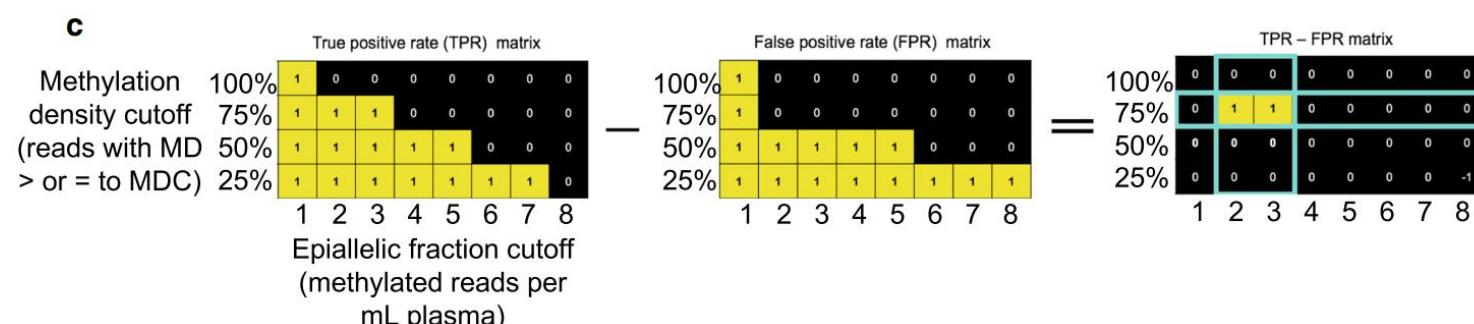
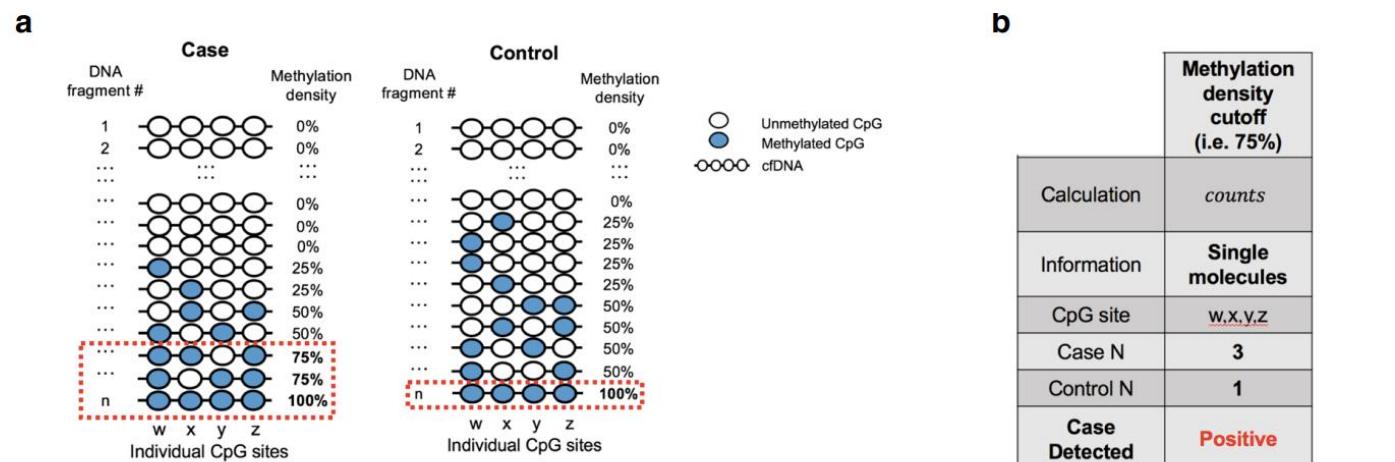
METHODOLOGY

Open Access

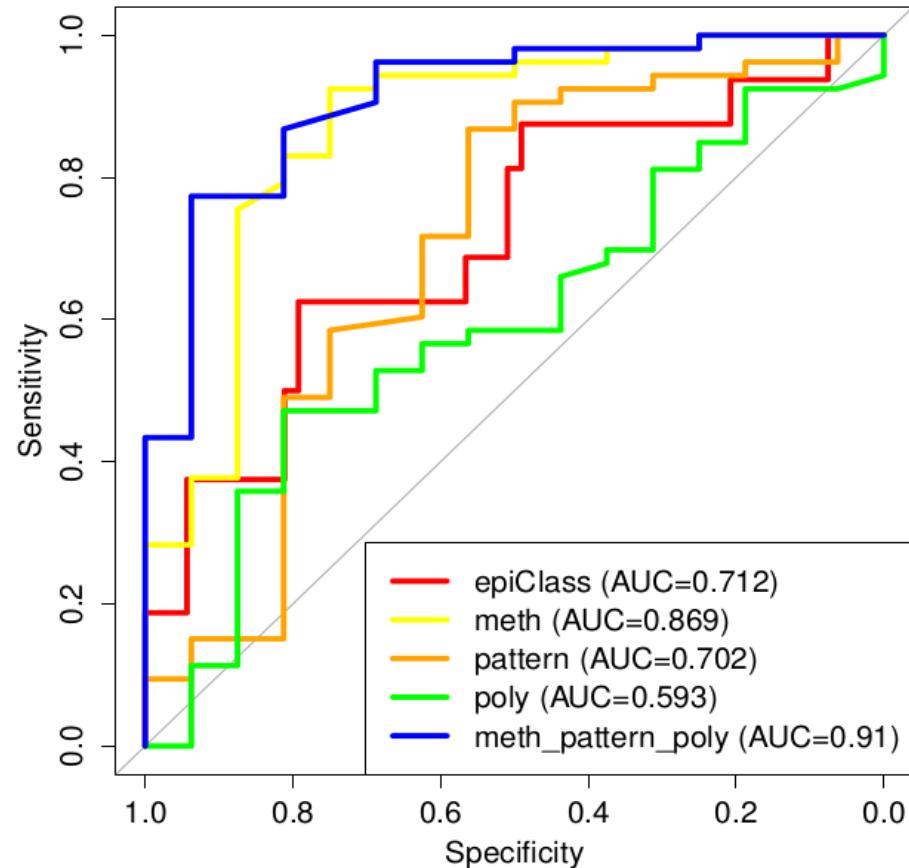


Leveraging locus-specific epigenetic heterogeneity to improve the performance of blood-based DNA methylation biomarkers

Brendan F. Miller¹, Thomas R. Pisanic II^{2*}, Gennady Margolin¹, Hanna M. Petrykowska¹, Pornpat Athamanolap⁵, Alexander Goncearenco¹, Akosua Osei-Tutu³, Christina M. Annunziata³, Tza-Huei Wang^{2,4,5} and Laura Elnitski^{1*}



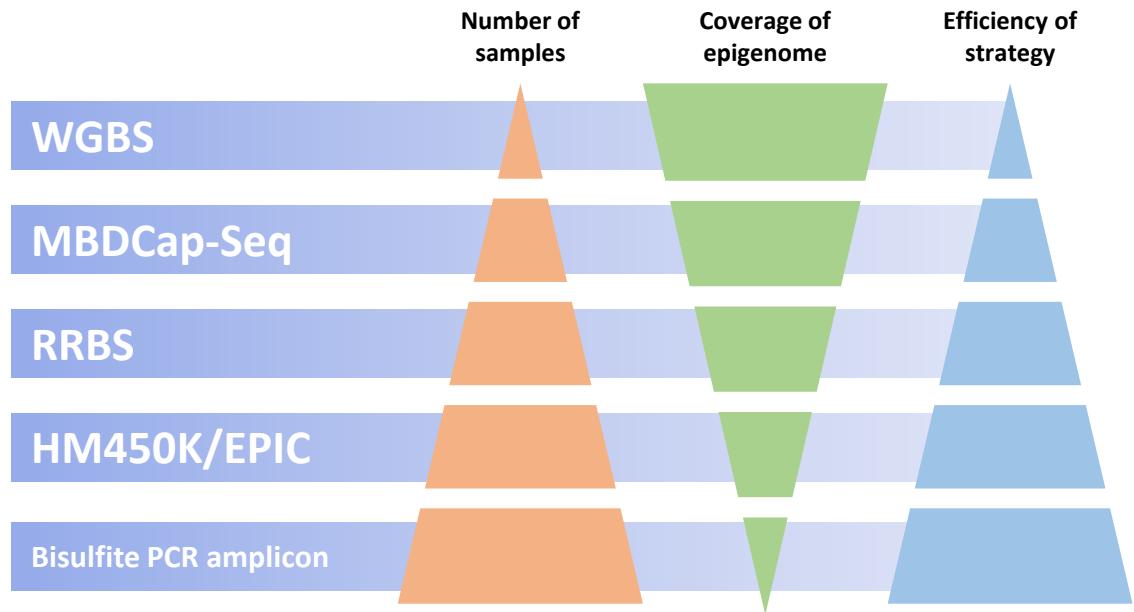
The power of combination of DNA methylation, Pattern and EpiPolymorphsim score for diagnosis of breast cancer using cfDNA (AUC=0.91)



methOnly	PC63_ZNF154::chr19:58220437-58220516_24
	PC66_UBD::chr6:29521527-29521604_11
	PC60_NBPF8::chr1:147775565-147775637_4
	PC51_FERD3L::chr7:19184932-19184993_49
	PC59_TACC1::chr8:38627860-38627915_38
patternOnly	PC46_intergenic::chr8:57069882-57069948_67
	PC63_ZNF154_TTTCTTTTTT
	PC54_POU4F2_TTTCTTTTTT
	PC49_STK3_CTTTTTC
	PC51_FERD3L_TTTTTCTT
epiClass	PC47_CYP26A1_TTTTCC
	PC66_UBD_CTTTTTTTTT
	PC63_ZNF154::chr19:58220437-58220516_24
	PC66_UBD::chr6:29521527-29521604_11
	PC60_NBPF8::chr1:147775565-147775637_4
polyOnly	PC51_FERD3L::chr7:19184932-19184993_49
	PC59_TACC1::chr8:38627860-38627915_38
	PC46_intergenic::chr8:57069882-57069948_67
	PC47_CYP26A1
	PC51_FERD3L
meth_pattern_poly	PC54_POU4F2
	PC49_STK3
	PC60_NBPF8
	PC59_TACC1
	PC46_intergenic

Conclusion

- We succeed to build up the breast cancer DNA methylation panel.
- The breast cancer panel of DNA methylation biomarkers is feasible for diagnosis of both tissue and liquid biopsy.
- The combine DNA methylation status of different CpGs or with the other features such as methylated patterns and epi-polymorphism showed better performance (AUC = 0.91) than a single one.



The best performance amplicons in the panel

order	name	rank
1	PC63_ZNF154::chr19:58220437-58220516_24	1.891429
2	PC66_UBD::chr6:29521527-29521604_11	2.46
3	PC60_NBPF8::chr1:147775565-147775637_4	3.405
4	PC51_FERD3L::chr7:19184932-19184993_49	5.479643
5	PC59_TACC1::chr8:38627860-38627915_38	6.851071
6	PC46_intergenic::chr8:57069882-57069948_67	7.403571

Clinical translation II, 3

Prognostic epigenetic biomarkers for prostate cancer mortality

Received: 24 March 2022

Revised: 2 August 2022

Accepted: 8 August 2022

DOI: 10.1002/ctm2.1030

RESEARCH ARTICLE

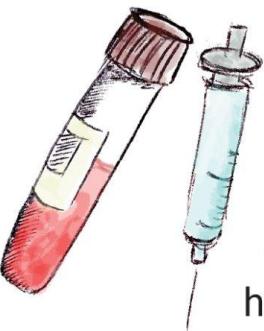


WILEY

Comprehensive methylome sequencing reveals prognostic epigenetic biomarkers for prostate cancer mortality

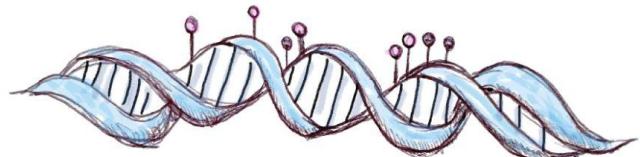
Ruth Pidsley^{1,2} | Dilys Lam¹ | Wenjia Qu¹ | Timothy J. Peters^{1,2} |
Phuc-Loi Luu^{1,2} | Darren Korbie³ | Clare Stirzaker^{1,2} | Roger J. Daly⁴ |
Phillip Stricker^{1,2,5} | James G. Kench^{1,6} | Lisa G. Horvath^{1,2,7,8} | Susan J. Clark^{1,2} 

Prostate cancer
is the second most
common cancer
diagnosed in men

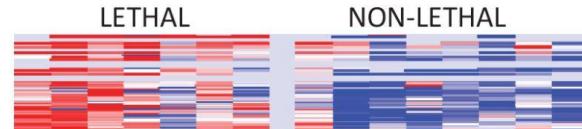


There is a critical need
for more biomarkers to
help guide management

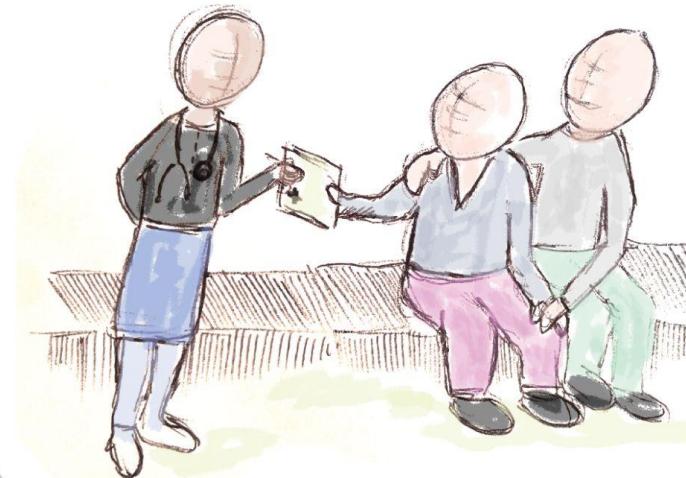
We know that **methylation markers**
on DNA are altered in prostate cancer



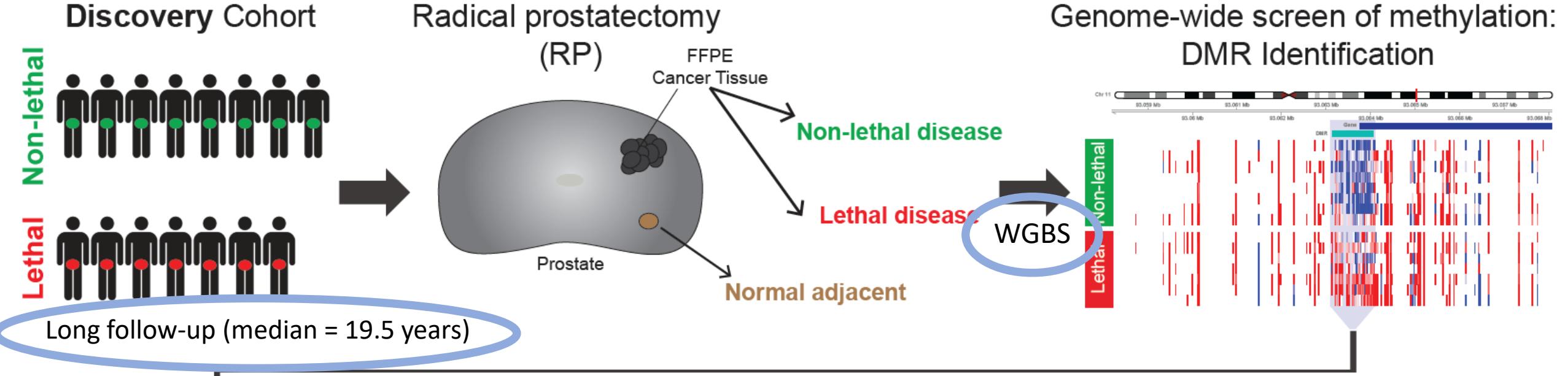
We compared lethal and non-lethal
prostate cancers and found prognostic
methylation markers on DNA



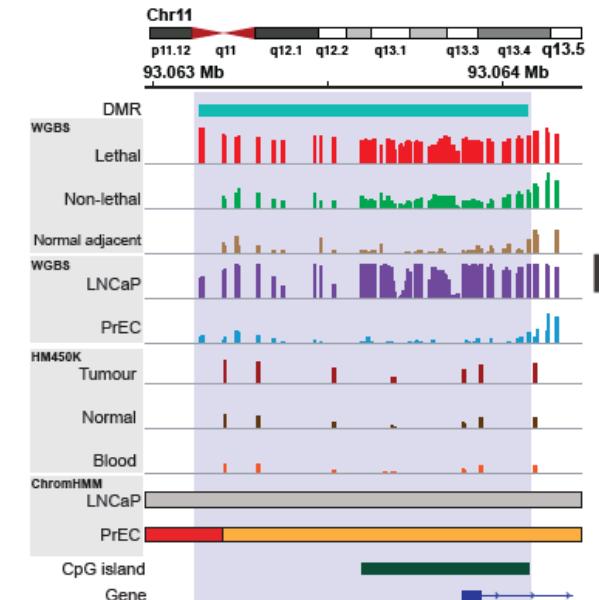
This **prognostic information** could
guide clinical decisions following a
diagnosis of prostate cancer



Credit to Dr Kate

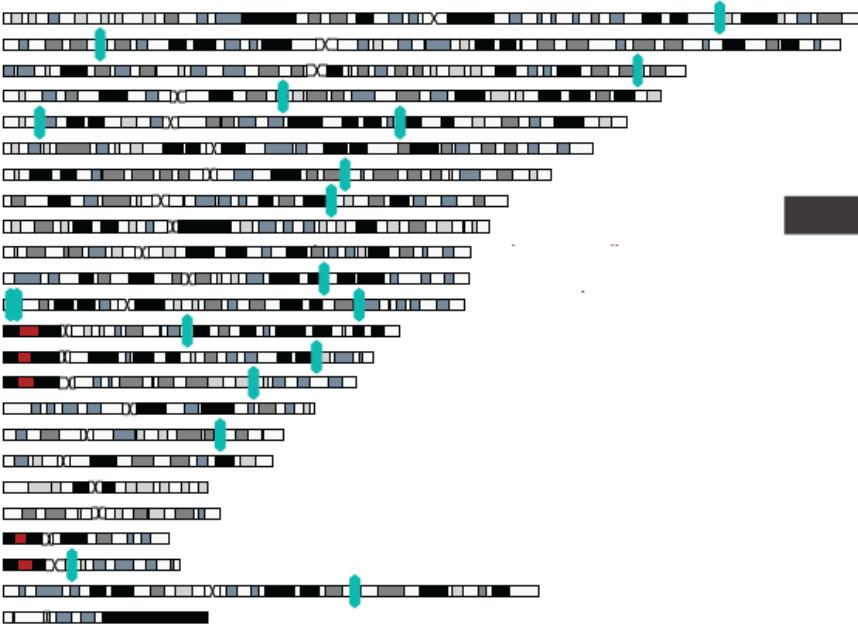


Biomarker selection

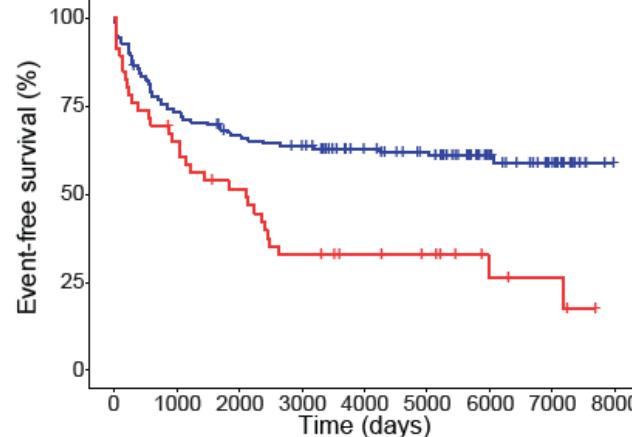


Comparison with in-house and public datasets

Targeted multiplex panel: 18 regions



Independent Validation RP Cohort (n = 185): Long follow-up (median = 15 years)

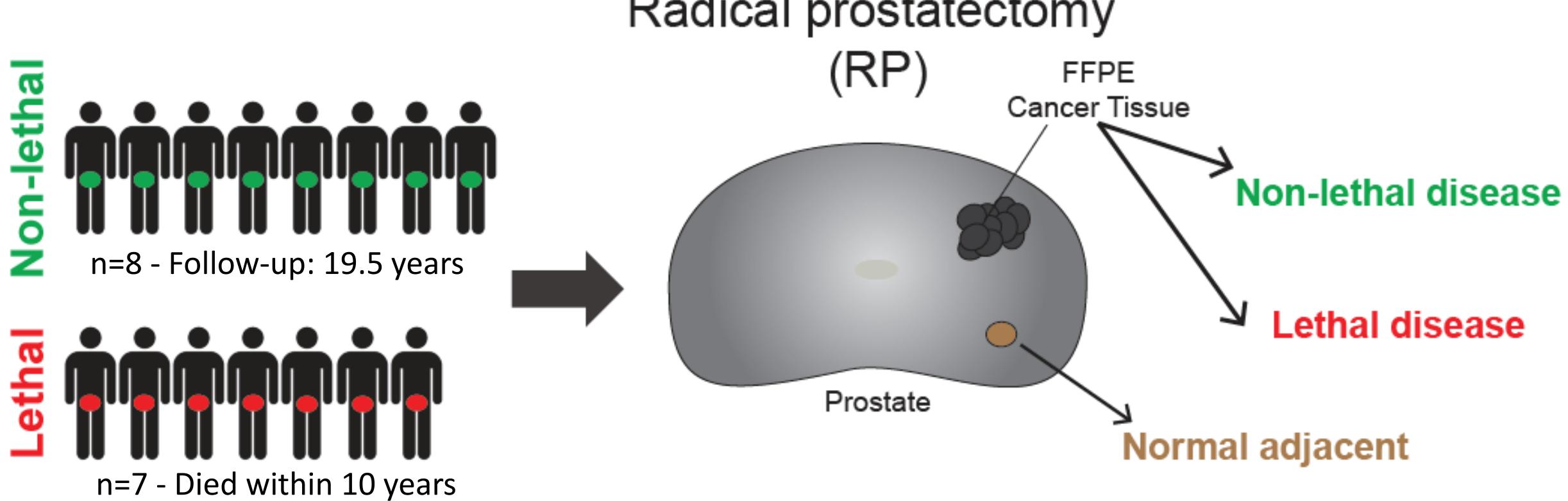


Survival analysis

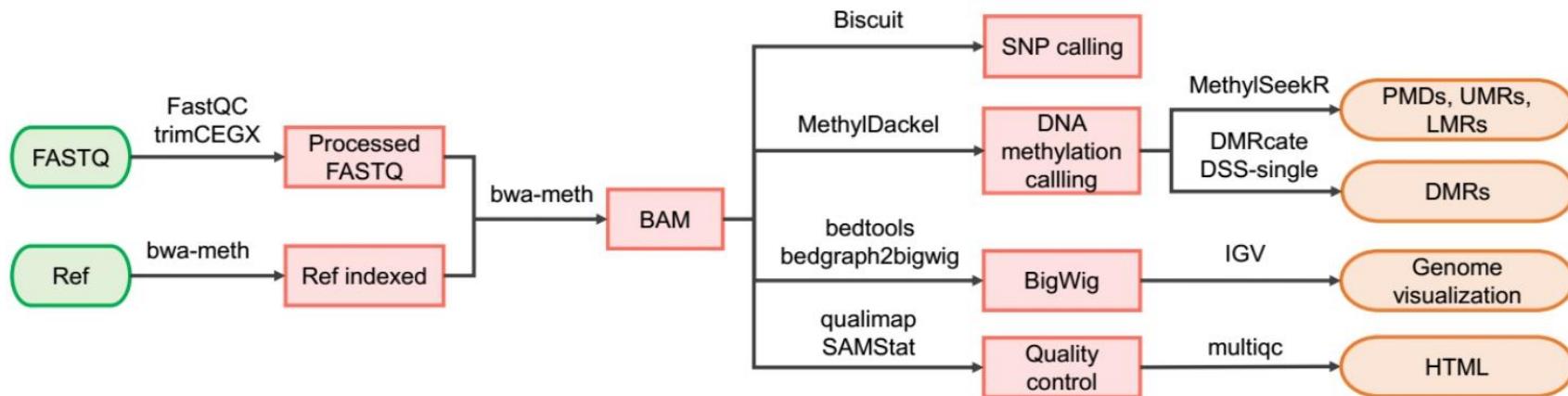
- Biochemical Recurrence
- Metastatic Relapse
- Prostate Cancer Death

Discovery

Whole Genome Bisulfite Sequencing (WGBS) on n=15 patients who had RP surgery 1989-2003, St Vincent's Hospital, Sydney:

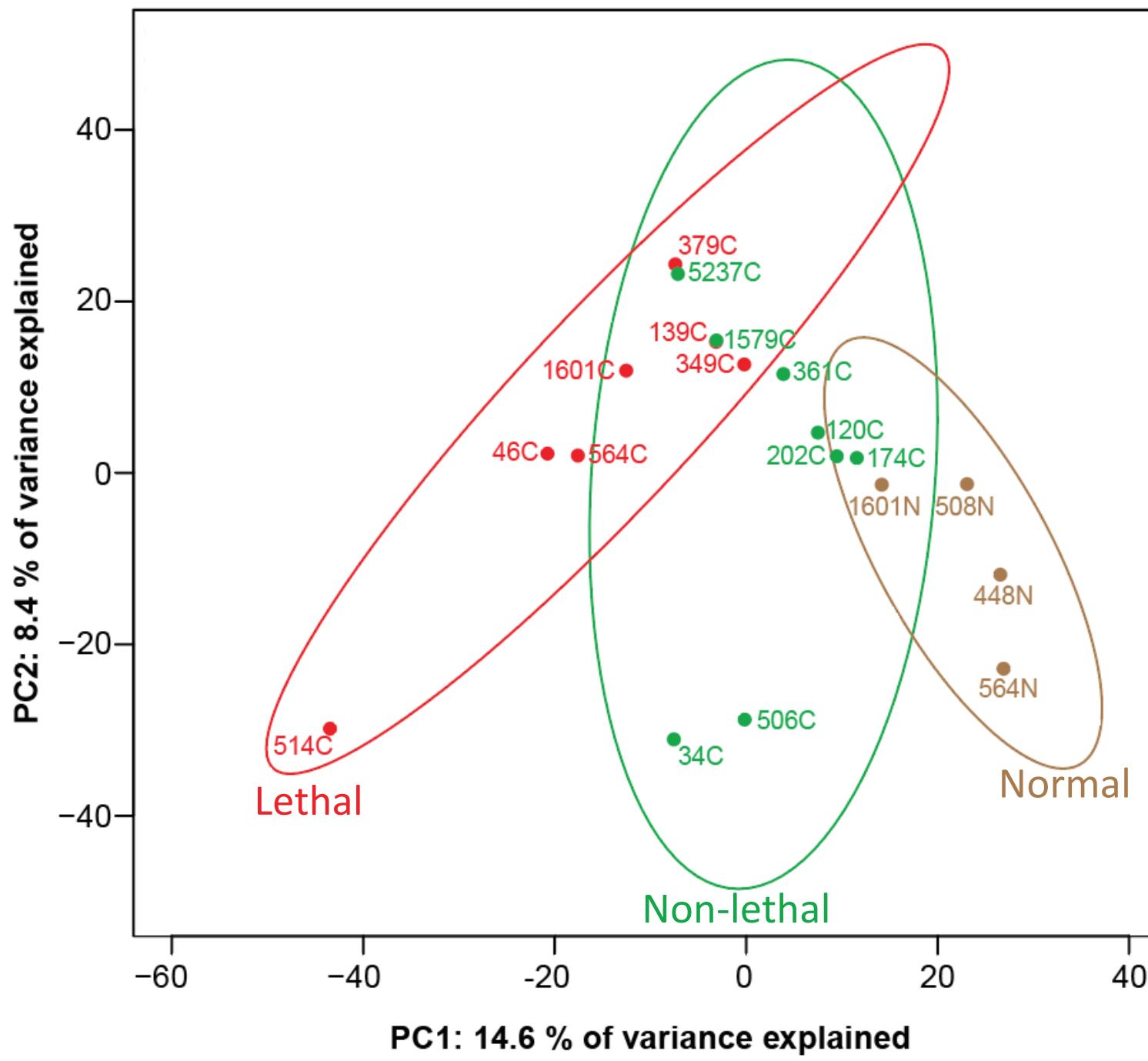


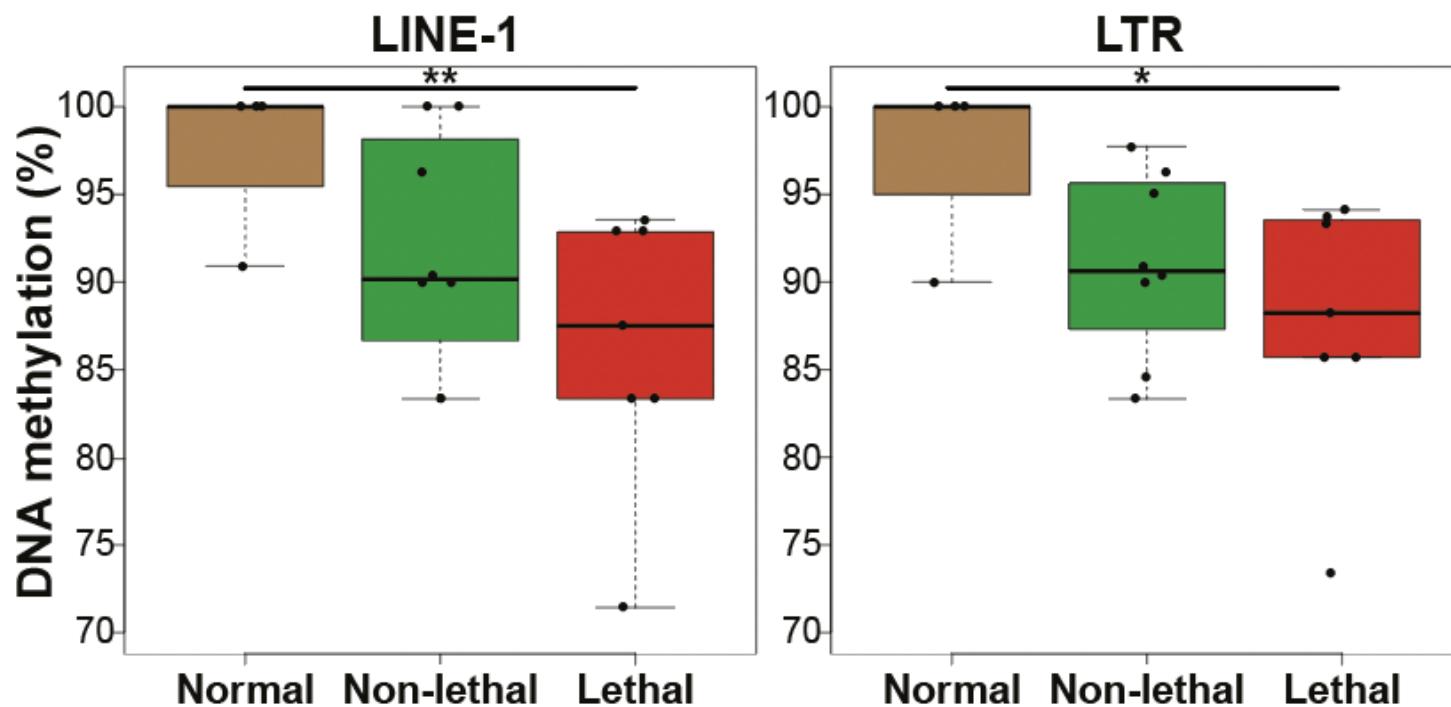
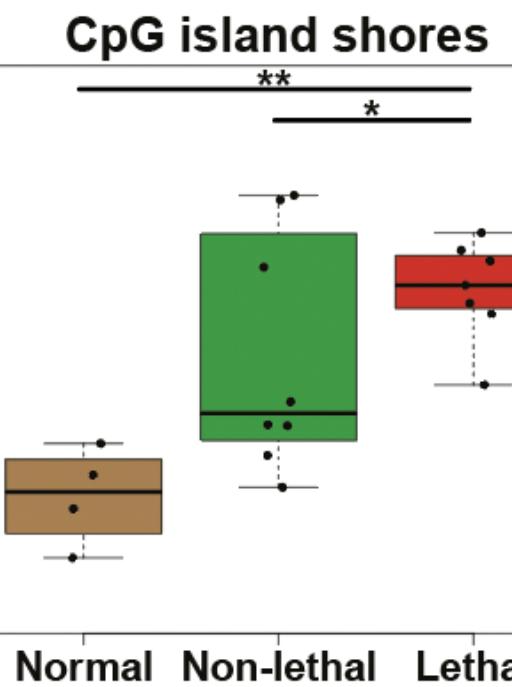
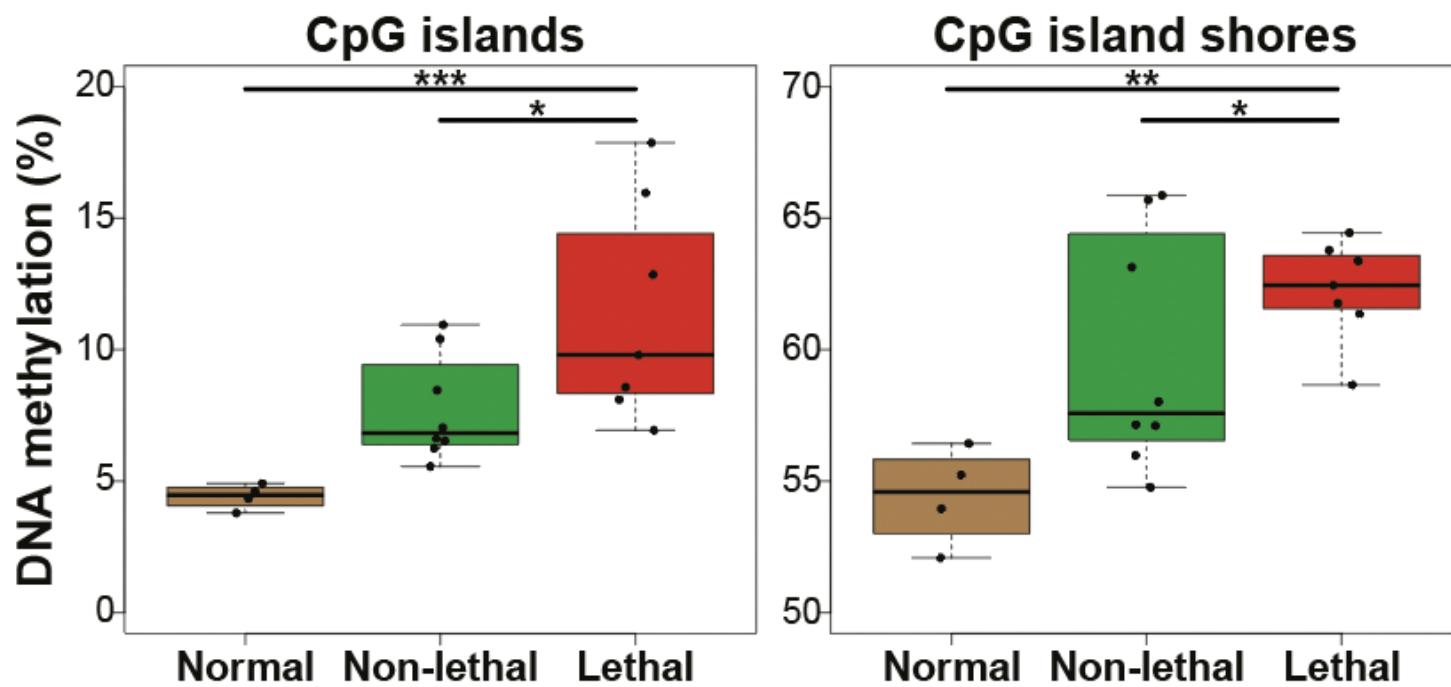
Whole Genome Bisulphite Sequencing pipeline



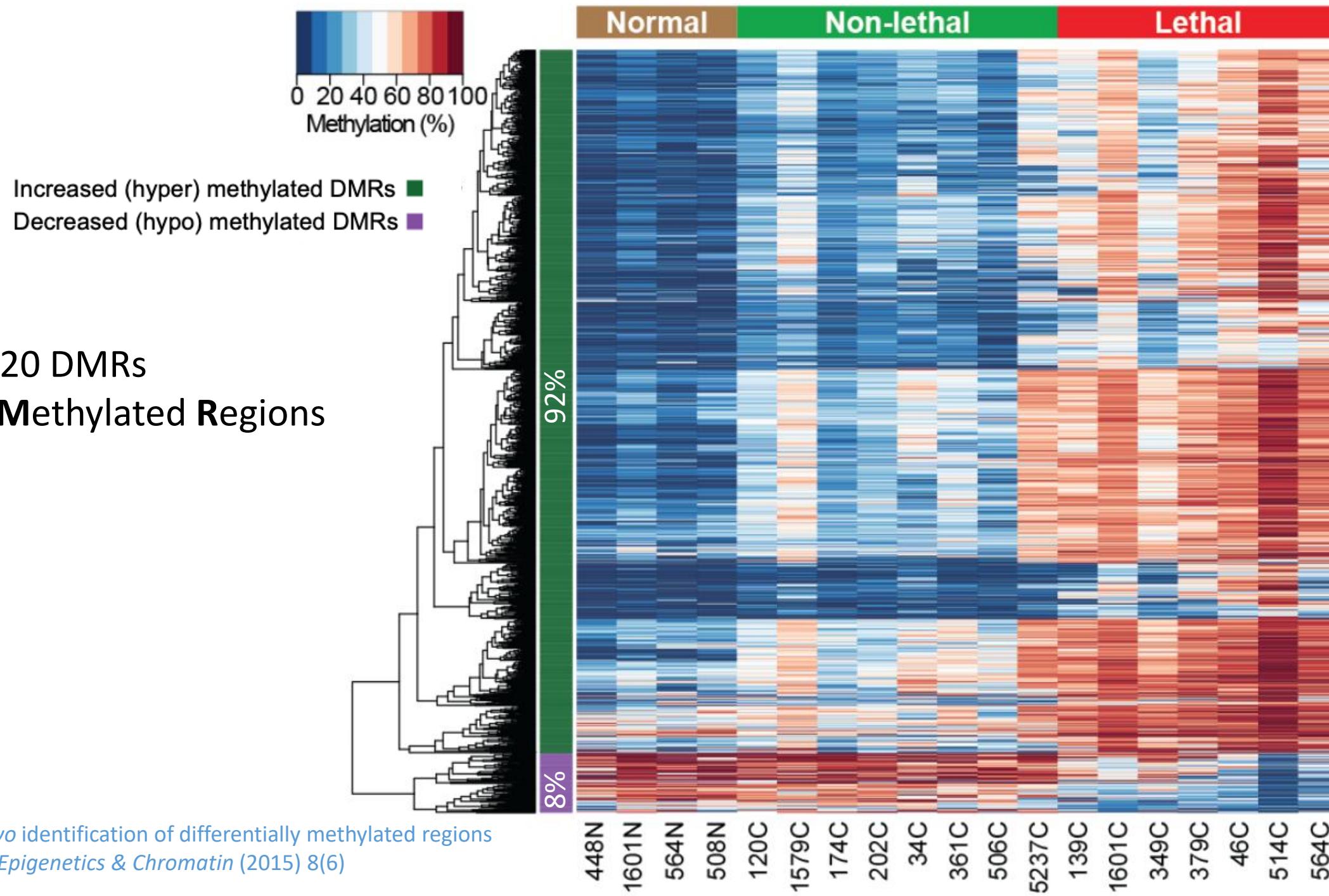
Package	Version	Link
FastQC	0.11.9	https://www.bioinformatics.babraham.ac.uk/projects/fastqc/
trimCEGX	1.0	https://github.com/luuloi/trim.paired.read
bwa-meth	0.10	https://github.com/brentp/bwa-meth
Biscuit	0.2.0	https://github.com/zwdzwd/biscuit
MethylDackel	0.2.0	https://github.com/dpryan79/MethylDackel
qualimap	2.2.1	http://qualimap.bioinfo.cipf.es/
SAMStat	1.08	http://samstat.sourceforge.net/
MethylSeekR	1.0	https://github.com/Bioconductor-mirror/MethylSeekR
DMRcate	2.2.3	https://bioconductor.org/packages/release/bioc/html/DMRcate.html
DSS-single	2.36.0	https://bioconductor.org/packages/release/bioc/html/DSS.html
multiqc	1.8	https://multiqc.info/

Figure 4. The WGBS pipeline and software.

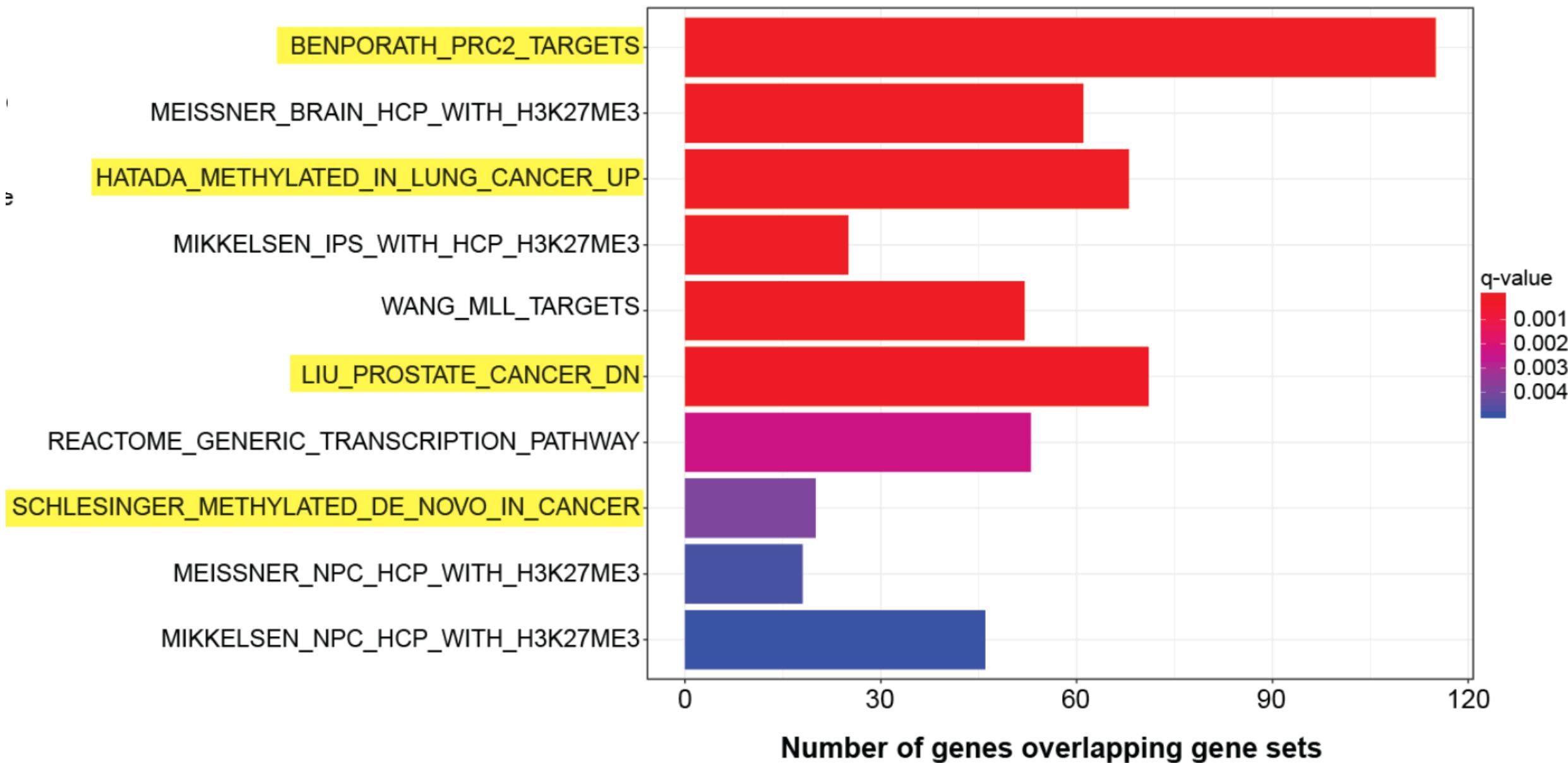




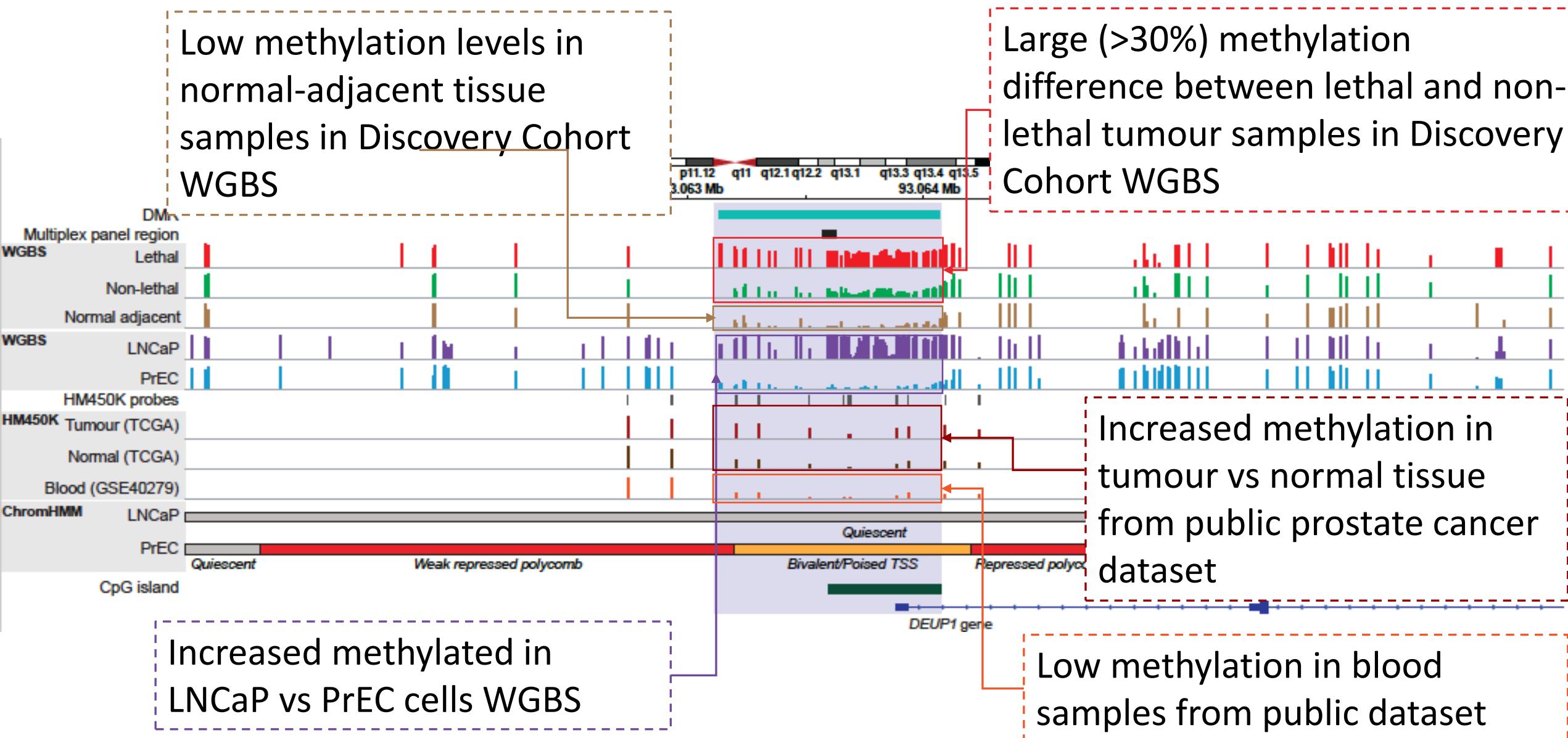
DMRcate: 1,420 DMRs Differentially Methylated Regions



Hypermethylated DMRs enriched for 298 gene sets. Top 10 MSigDB C2 collection:



DMR selection for validation



Targeted multiplex panel – 18 DMRs



	DMR no.	Genomic position (hg19)	Nearest protein coding gene
1	1	chr1:208132439-208132824	<i>CD34</i>
2	2	chr2:27958210-27958689	<i>AC074091.13</i>
3	3	chr3:184243657-184243936	<i>EPHB3</i>
4	4	chr4:81118427-81118588	<i>PRDM8</i>
5	5	chr5:10333634-10334055	<i>MARCH6</i>
6	6	chr5:115151283-115152645	<i>CDO1</i>
7	7	chr7:99155673-99157071	<i>ZNF655</i>
8	8	chr8:95246476-95246871	<i>CDH17</i>
9	9	chr11:93063135-93064069	<i>DEUP1</i>
10	10	chr12:1906206-1906676	<i>CACNA2D4</i>
11	11	chr12:3862069-3862497	<i>CRACR2A</i>
12	12	chr12:103311054-103311276	<i>PAH</i>
13	13	chr13:53312994-53313591	<i>CNMD</i>
14	14	chr14:90849492-90850589	<i>CALM1</i>
15	15	chr15:72564636-72565252	<i>PARP6</i>
16	16	chr17:62773682-62777796	<i>LRRC37A3</i>
17	17	chr22:19742681-19743728	<i>TBX1</i>
X	18	chrX:102000717-102001518	<i>BHLHB9</i>

Lam, D., et al. Comprehensive evaluation of targeted multiplex bisulphite PCR sequencing for validation of DNA methylation biomarker panels. *Clin Epigenet* (2020) 12(90)

Validation

Multiplex Bisulfite PCR Sequencing (MBPS) on n=185 patients who had RP surgery 1997-2003 at St Vincent's Hospital, Sydney

Characteristic	
Number of patients	185
Age at RP, mean \pm s.d. (range)	61.9 \pm 5.8 (46 - 73)
ISUP Grade Groups	
2 (Gleason score 3 + 4), n (%)	119 (64.3)
3 (Gleason score 4 + 3), n (%)	34 (18.4)
4 (Gleason score 8), n (%)	19 (10.3)
5 (Gleason score 9, 10), n (%)	13 (7.0)
Pre-operative PSA (ng/mL), mean \pm s.d. (range)	10.3 \pm 7.3 (1.6 - 63.0)
PSA <10 ng/mL, n (%)	115 (62.2)
PSA \geq 10 ng/mL, n (%)	70 (37.8)
Pathological T-category	
\leq pT2, n (%)	89 (48.1)
\geq pT3, n (%)	96 (51.9)
Surgical Margin Status ²	
Negative, n (%)	91 (49.2)
Positive, n (%)	93 (50.3)
Follow up (years), median (range)	15 (0.8 - 22)
Clinical outcome	
Biochemical recurrence, n (%)	86 (46.5)
Metastatic relapse, n (%)	25 (13.5)
Prostate cancer specific mortality, n (%)	16 (8.6)

Survival analysis

Survival end-points:

- Biochemical Recurrence
- Metastatic Relapse
- Prostate Cancer Specific Mortality

1. Univariable analysis: clinicopathological - Log-rank and Cox regression ([Grade, Stage, PSA, Margins](#))

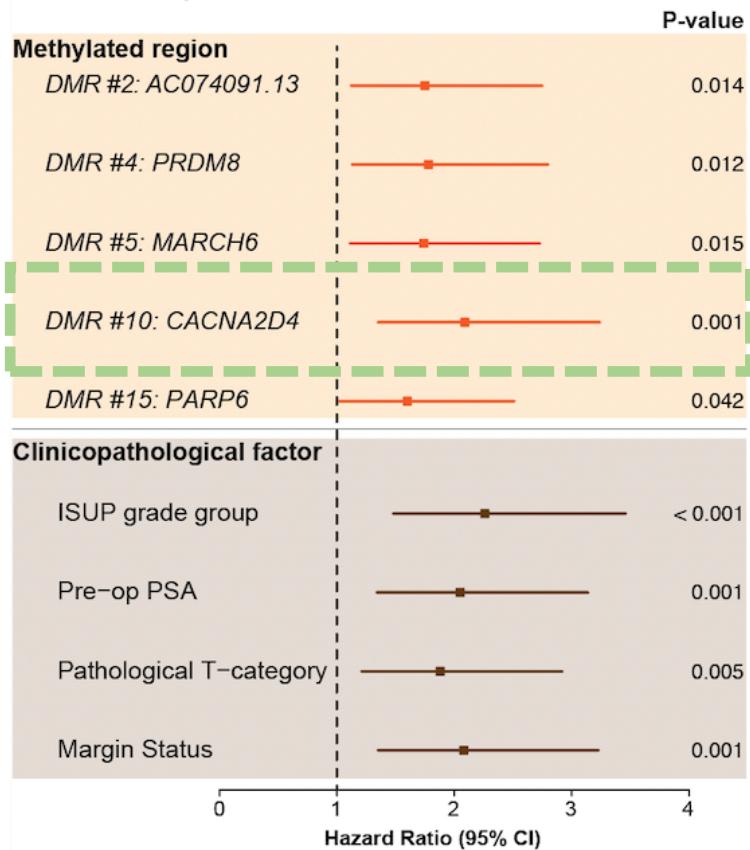
2. Univariable analysis: methylation - Log-rank and Cox regression ([DMRs](#))

3. Multivariable analysis: clinicopathological and methylation - Multivariable Cox regression using the SES feature selection algorithm ([MXM package in R](#))

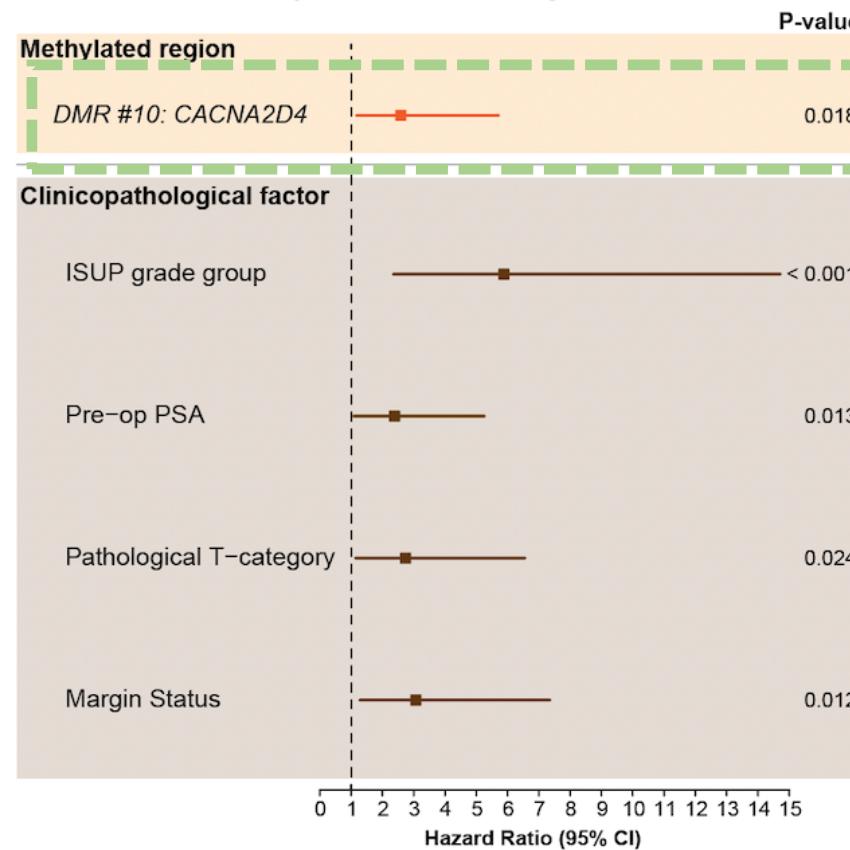
2. Univariable analysis: methylation

Univariable Cox regression

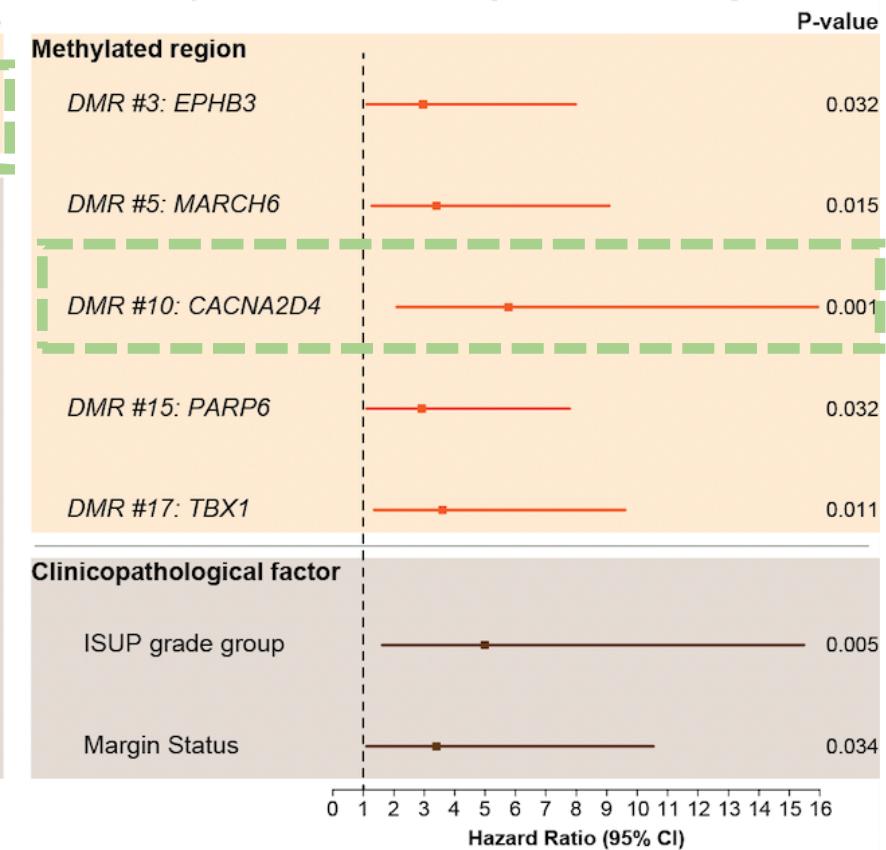
i) Biochemical recurrence



ii) Metastatic relapse

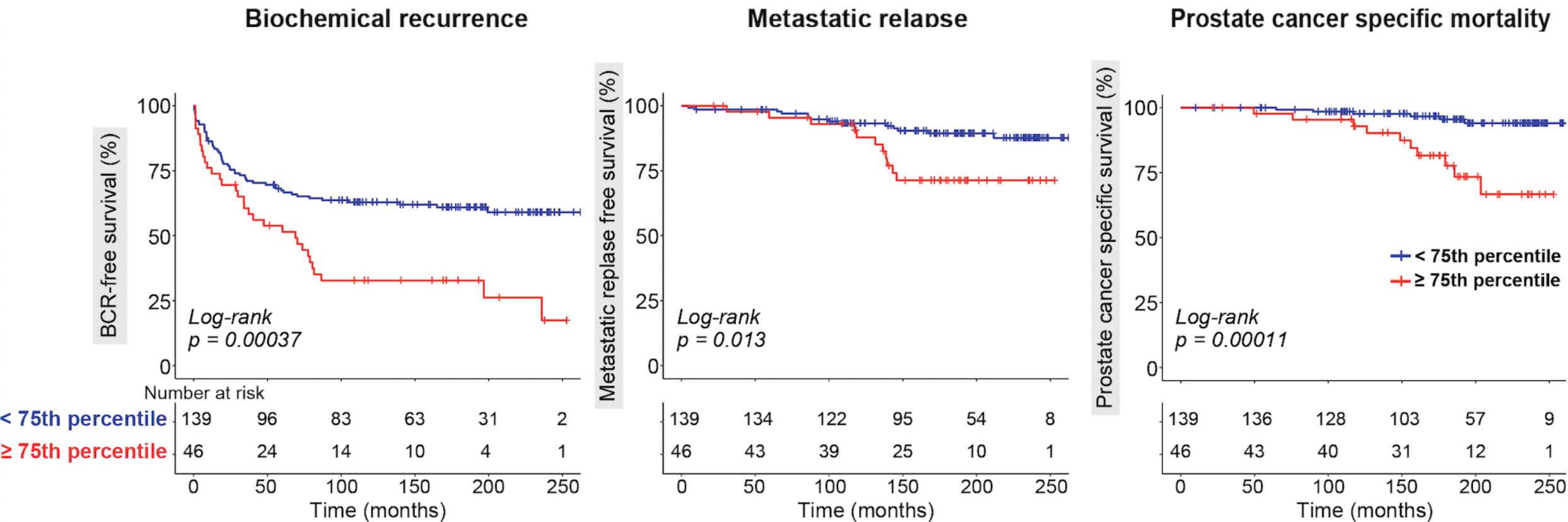


iii) Prostate cancer specific mortality



2. Univariable analysis: methylation

Kaplan Meier – CACNA2D4

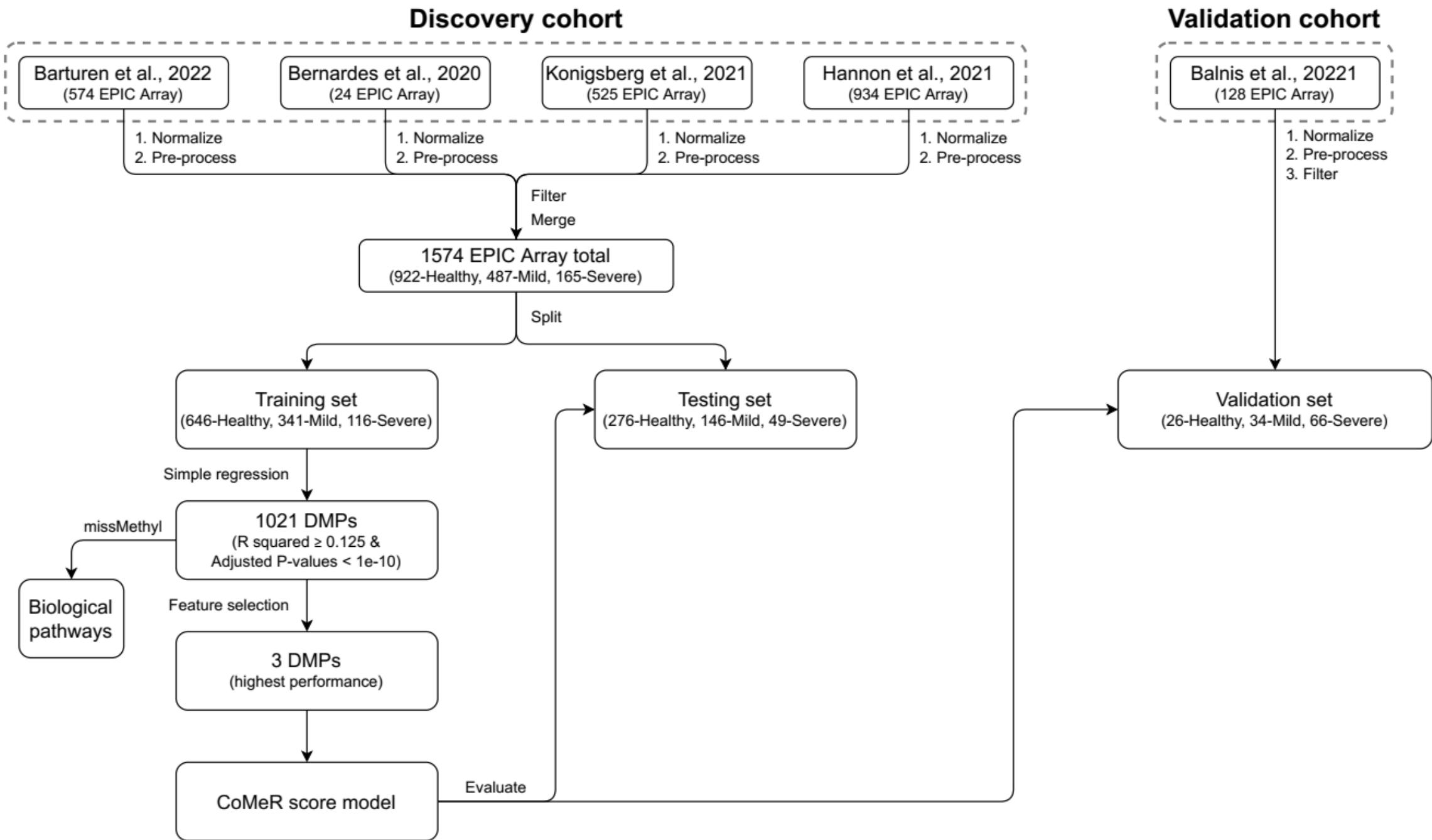


Clinical translation II, 4

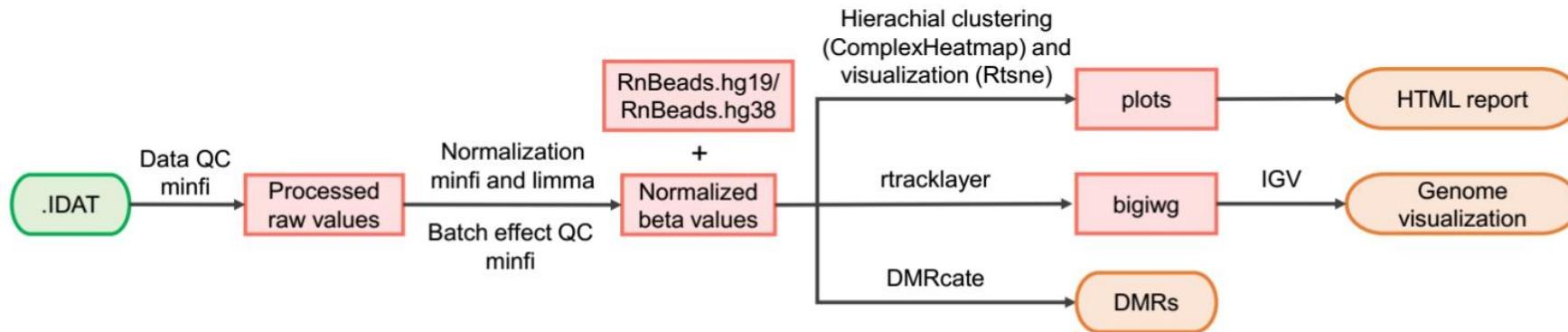
COVID19 DNA methylation risk (COMER) score for
severity classification

Aims

1. Development of COVID-19 Methylation risk (CoMeR) score for Severity
2. Validation and Characterization of CoMeR score
3. ShinyApp for prediction of CoMeR

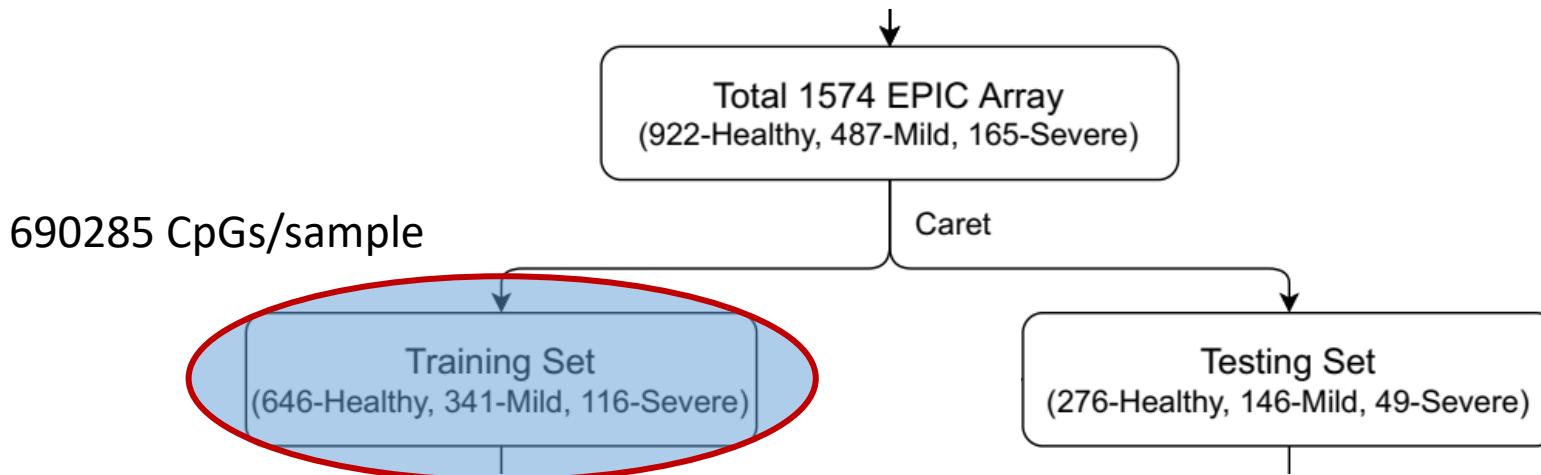


DNA Methylation Array (450K and EPIC/850K) pipeline

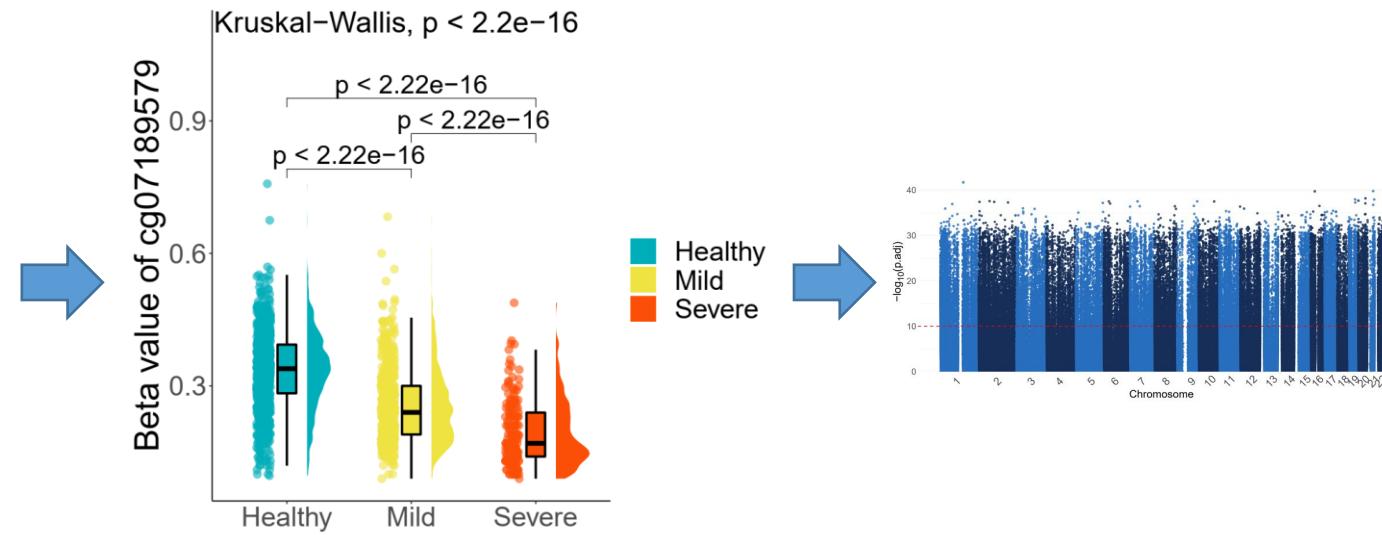


Package	Version	Link
minfi	1.34.0	http://bioconductor.org/packages/release/bioc/html/minfi.html
limma	3.44.3	https://bioconductor.org/packages/release/bioc/html/limma.html
RnBeads.hg19	1.20.0	https://bioconductor.org/packages/release/data/experiment/html/RnBeads.hg19.html
RnBeads.hg38	1.20.0	https://bioconductor.org/packages/release/data/experiment/html/RnBeads.hg38.html
Complex-Heatmap	2.4.3	https://www.bioconductor.org/packages/release/bioc/html/ComplexHeatmap.html
Rtsne	0.15	https://cran.r-project.org/web/packages/Rtsne/index.html
rtracklayer	1.48.0	https://bioconductor.org/packages/release/bioc/html/rtracklayer.html
DMRcate	2.2.3	https://bioconductor.org/packages/release/bioc/html/DMRcate.html

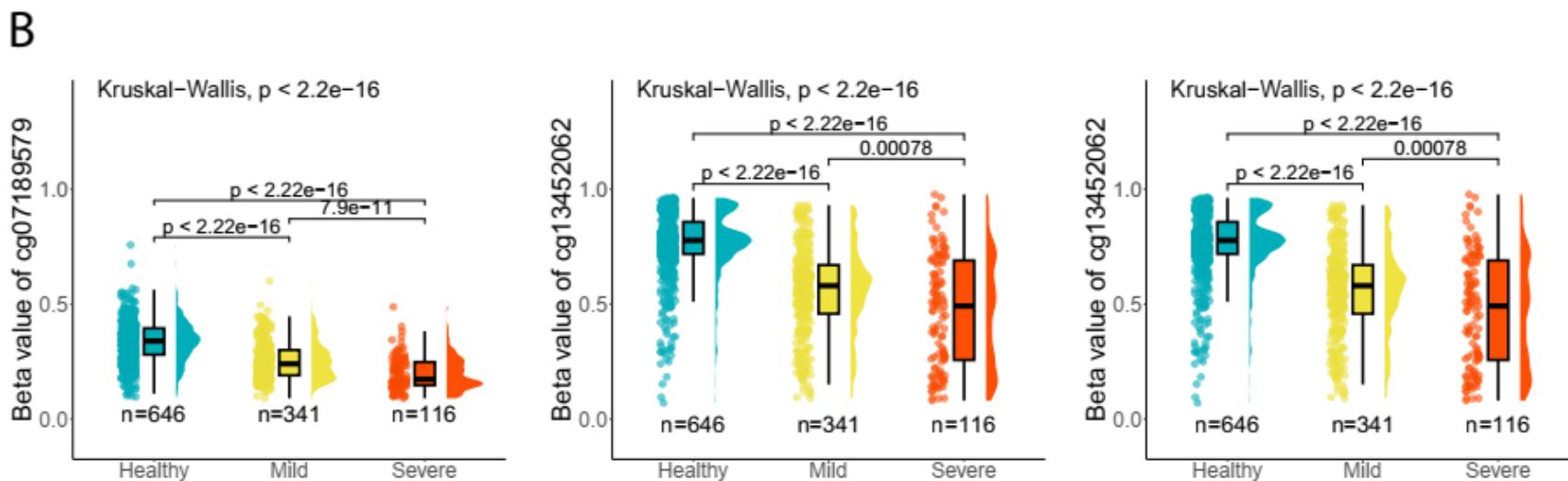
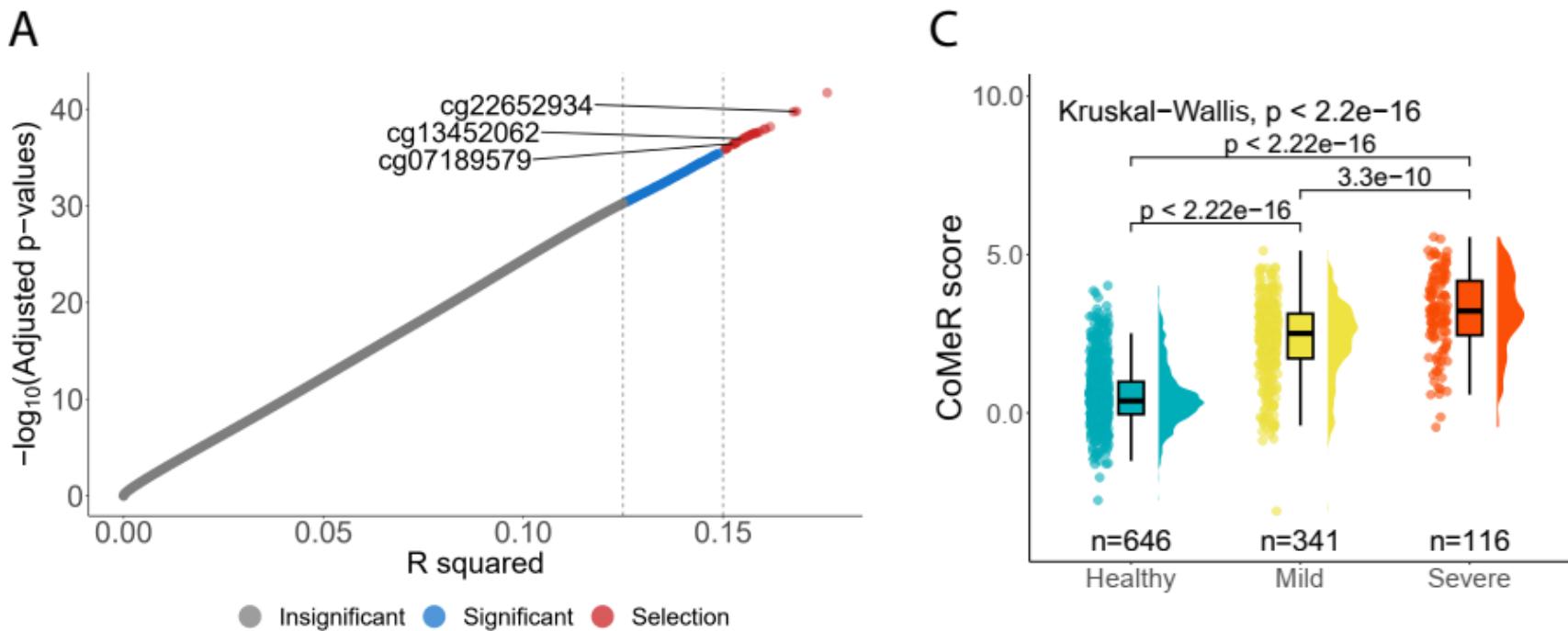
Figure 5. The DNA methylation array (Illumina Methylation 450K and 850K) pipeline and software.



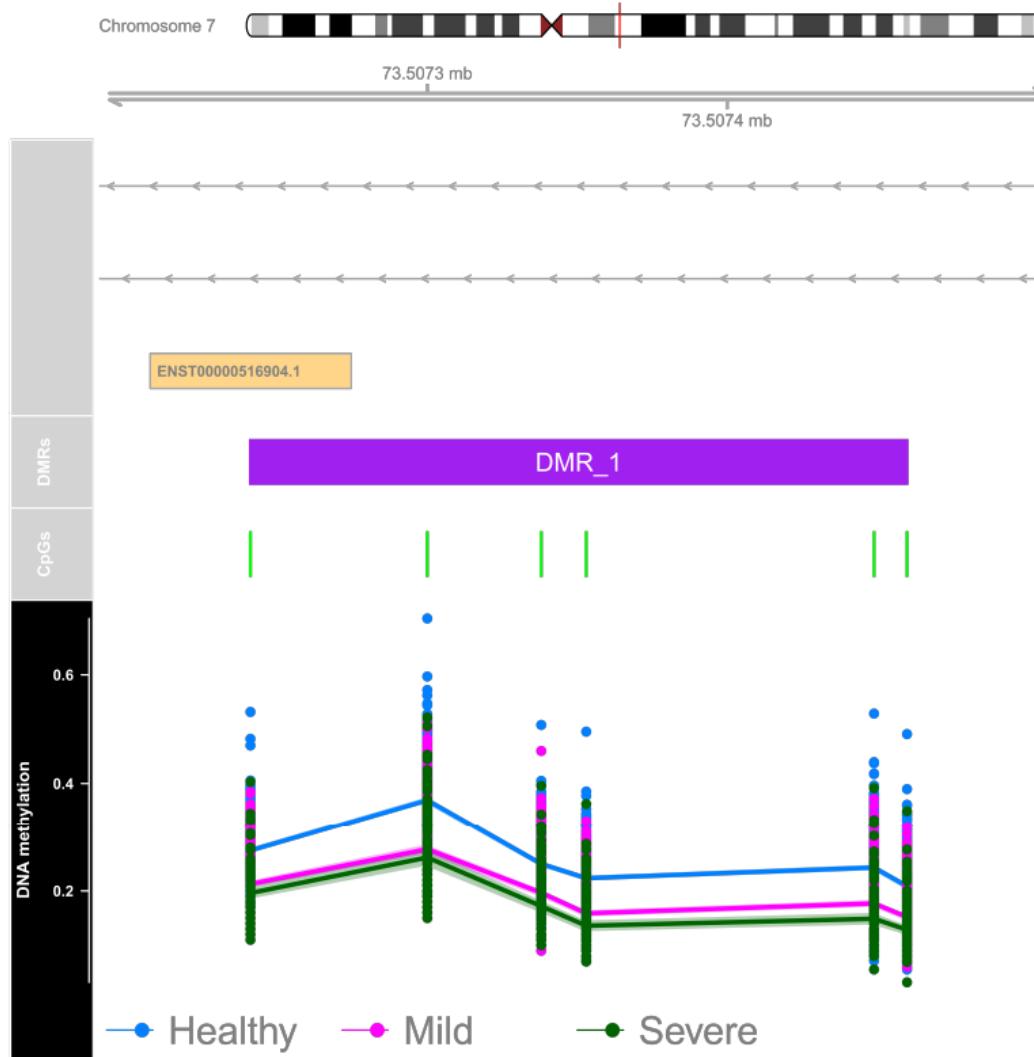
	sample1	sample2	...
cg1			
cg2			
...			



Differentially Methylated Probe (DMP)

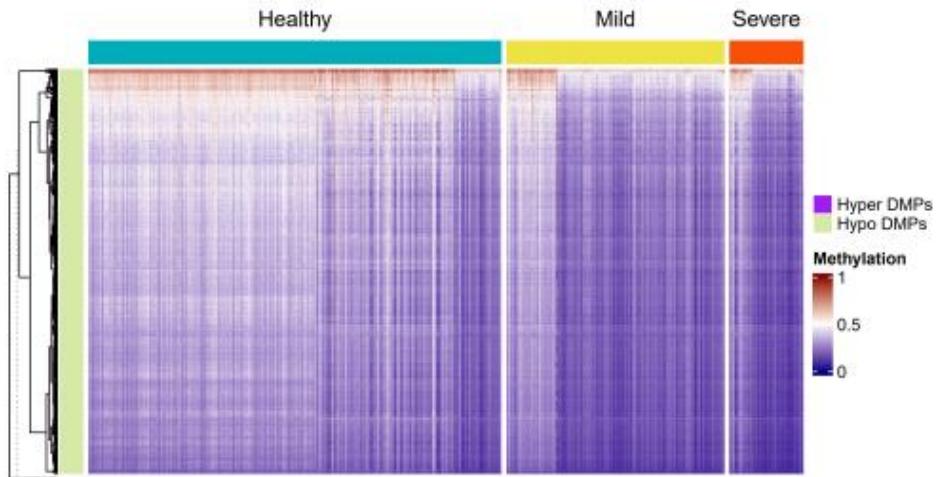
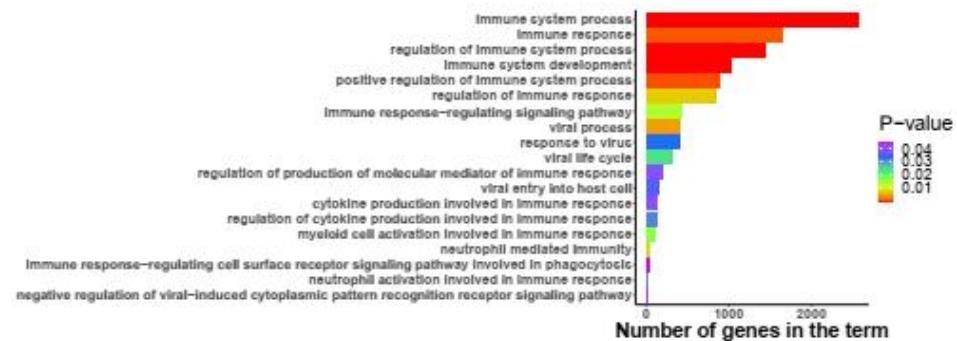
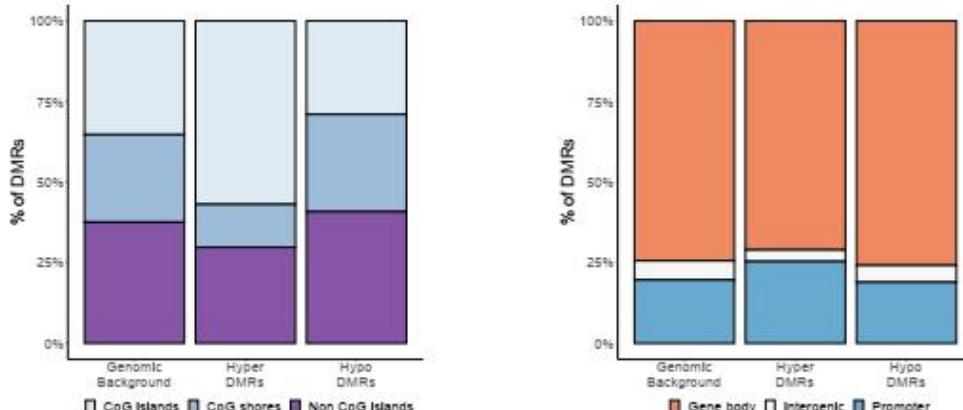
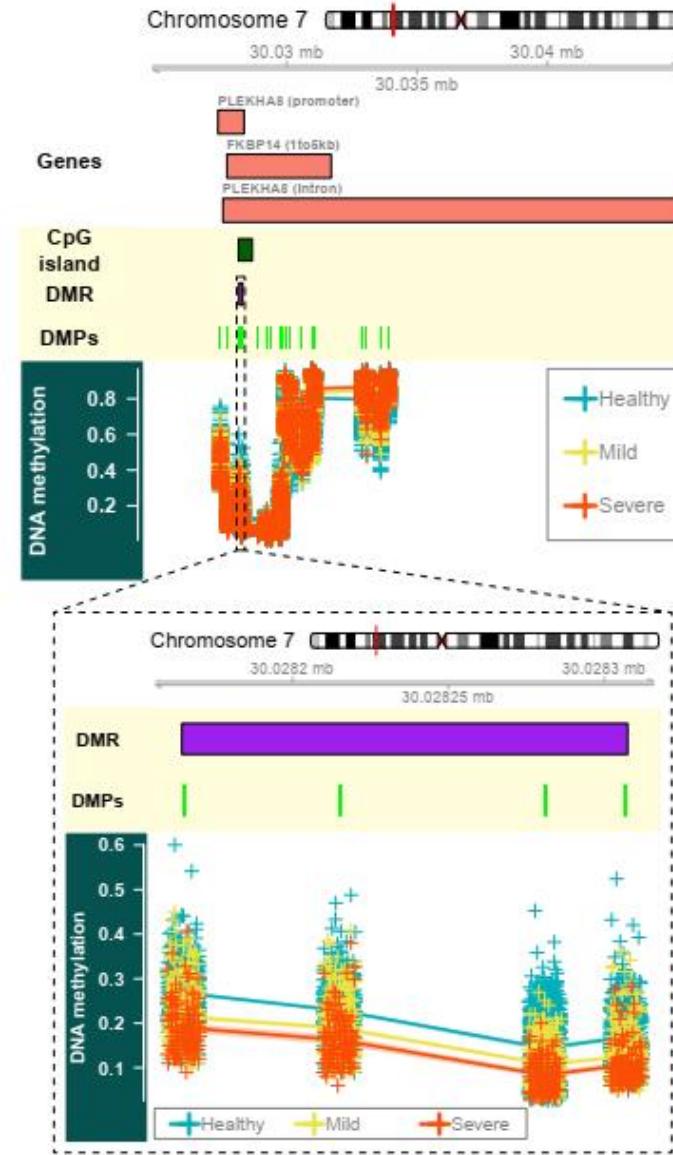


Differential Methylation Region (DMR) ~ 500

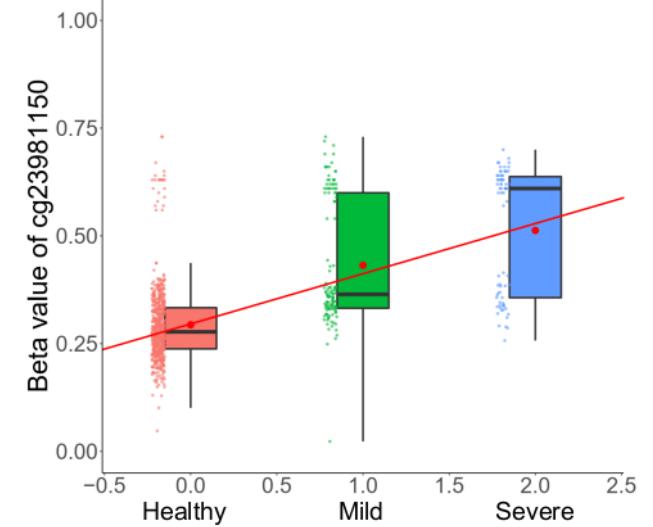
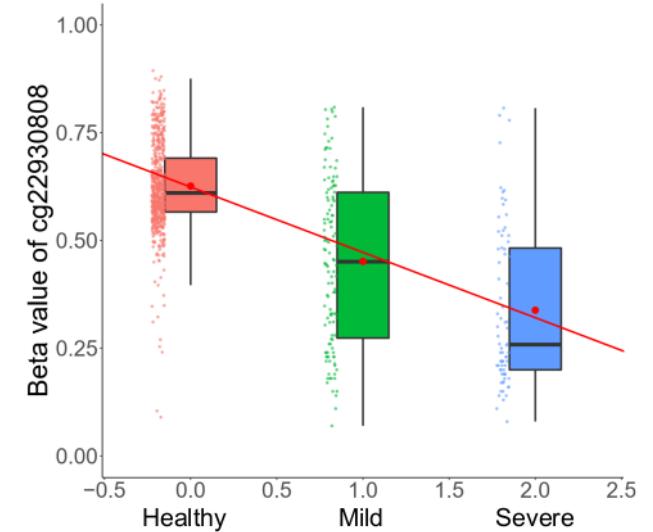
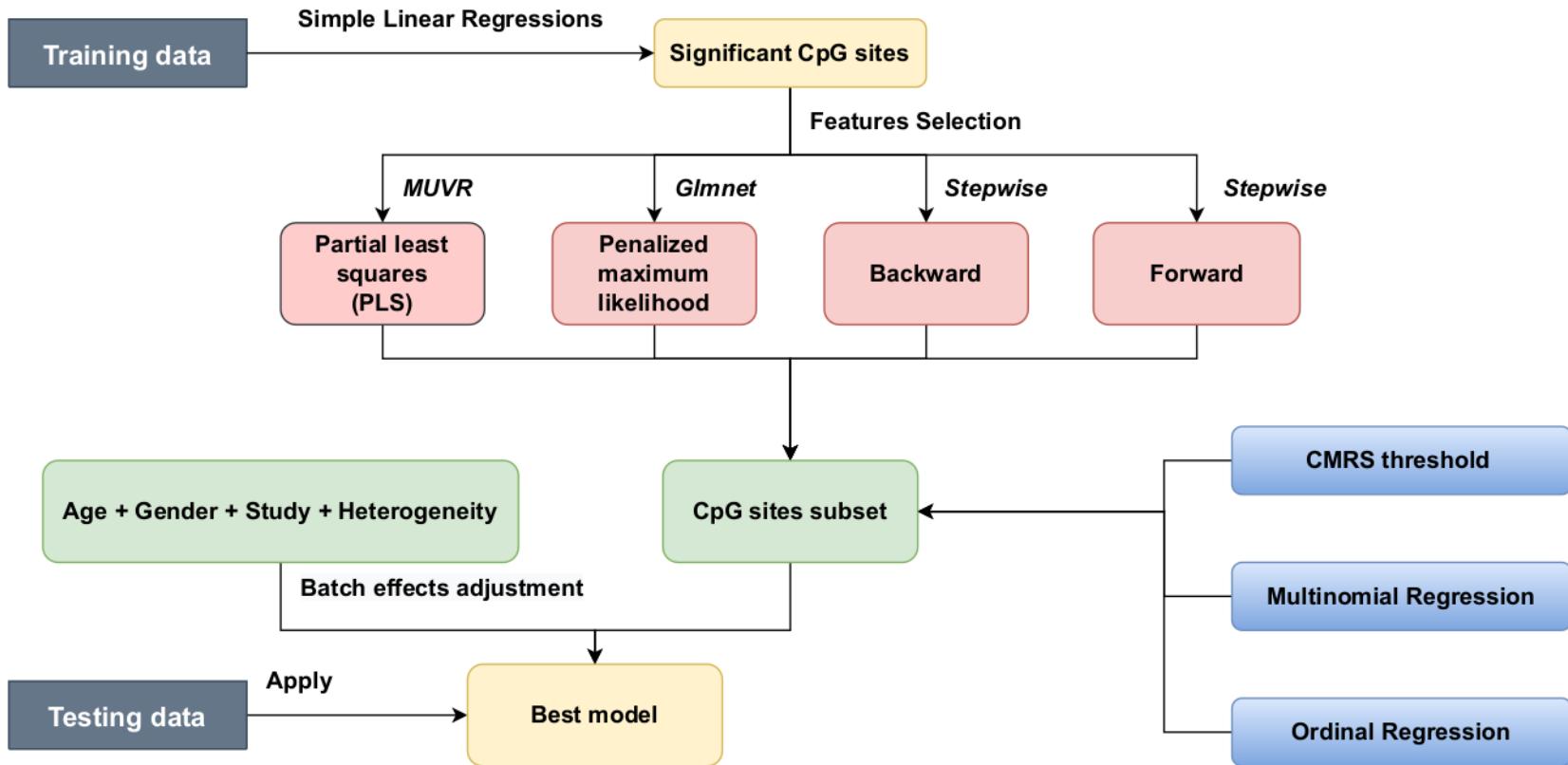


A

1021 probes
(1 hypermethylated)

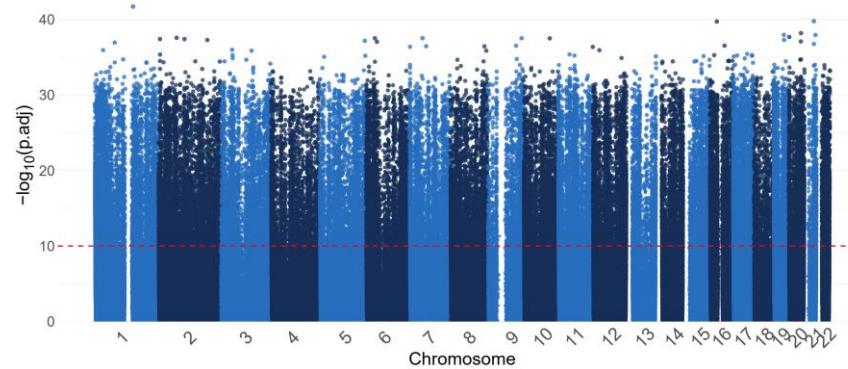
**B****C****D**

Feature selection workflow



Identify DNA methylation biomarker for severity

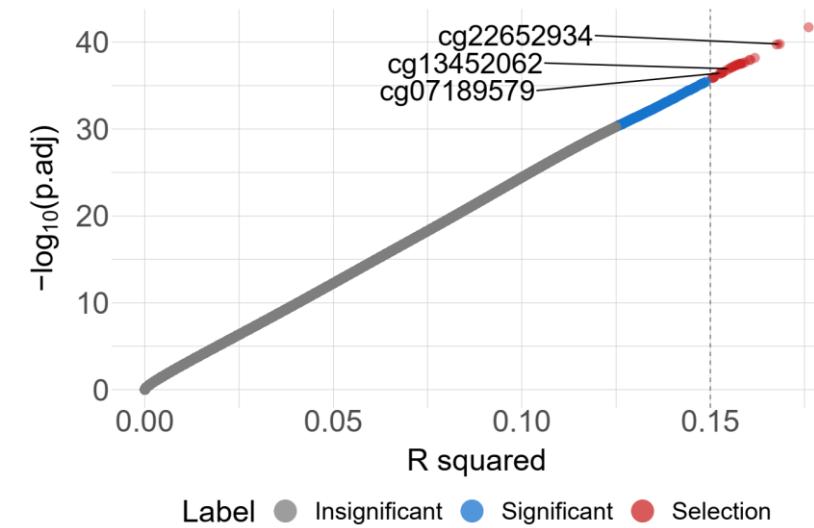
CoMeR score



R-squared



1021 DMPs



Label ● Insignificant ● Significant ● Selection

CoMeR score
for Severity patients

Build model

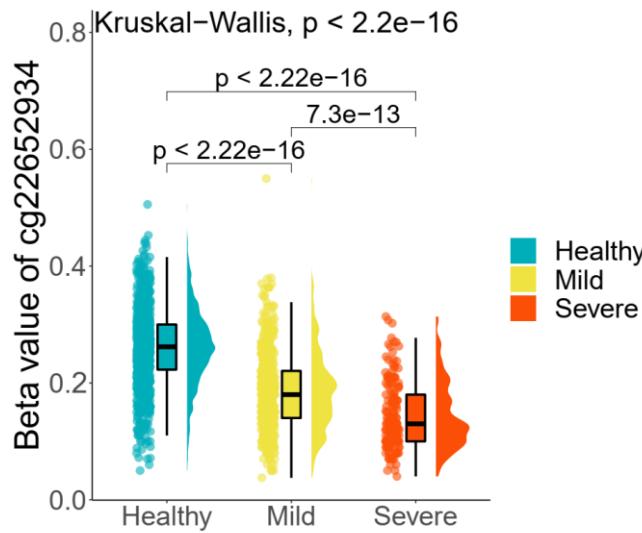
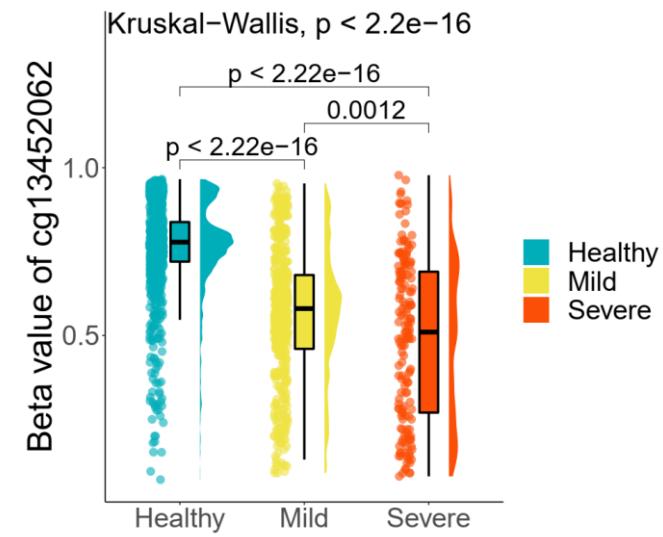
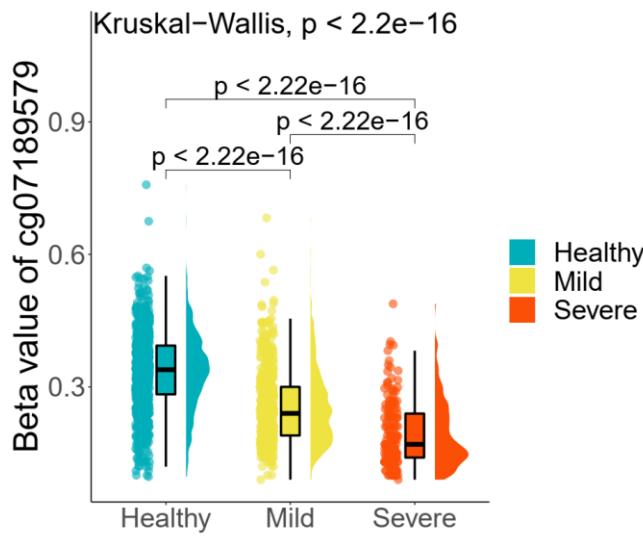


3 importance DMPs

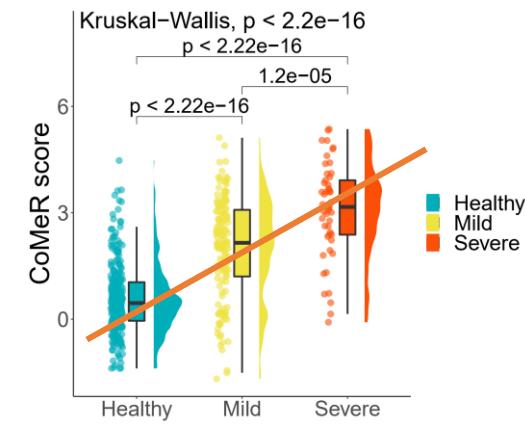
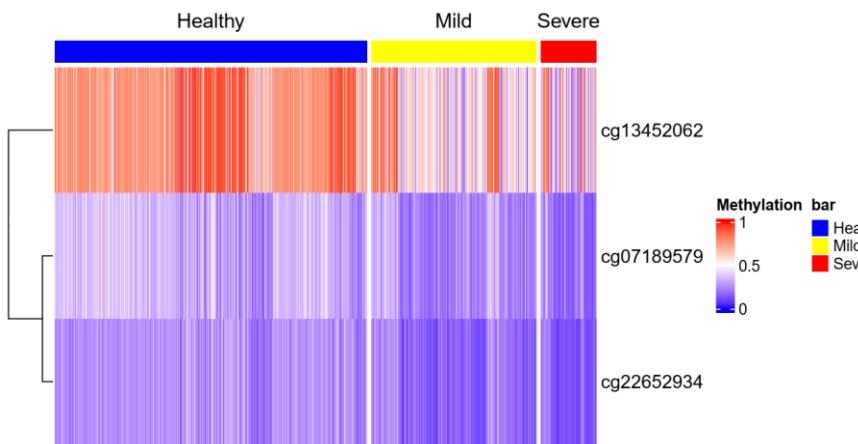


Feature Selection

Feature selection gives 3 importance DMPs



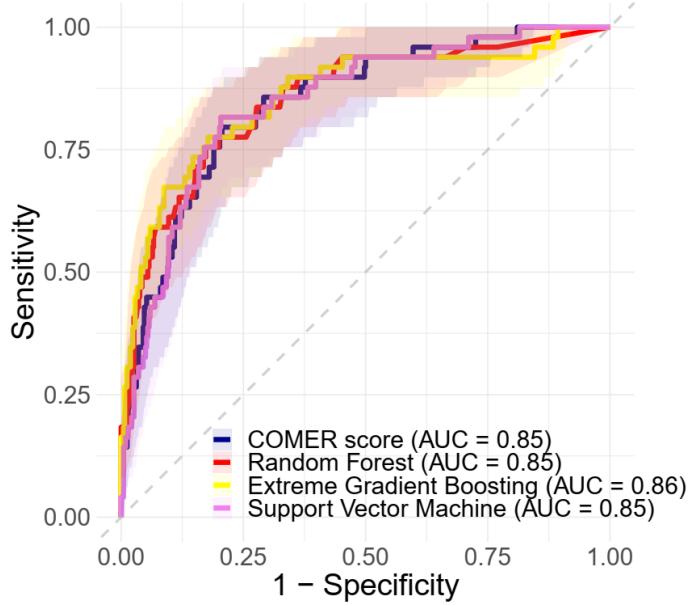
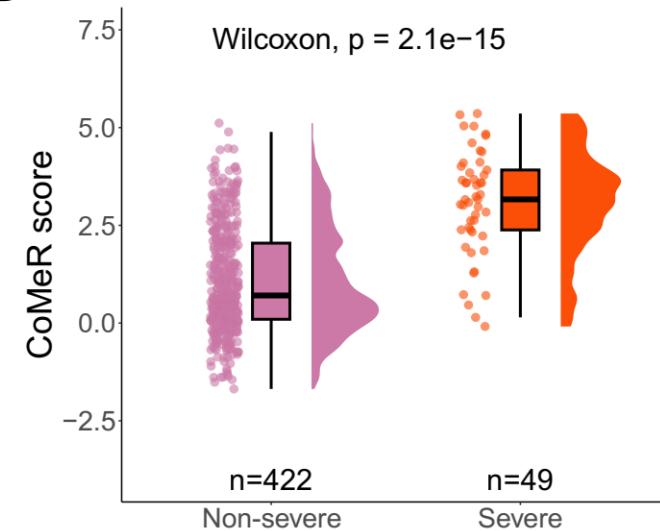
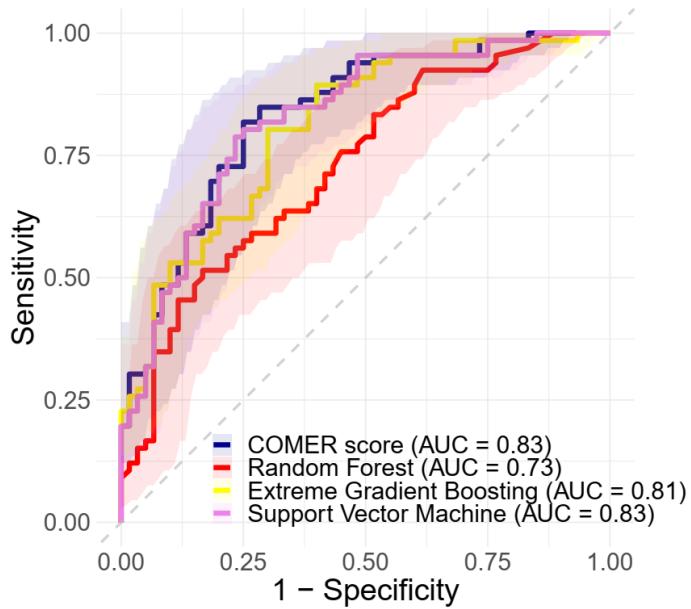
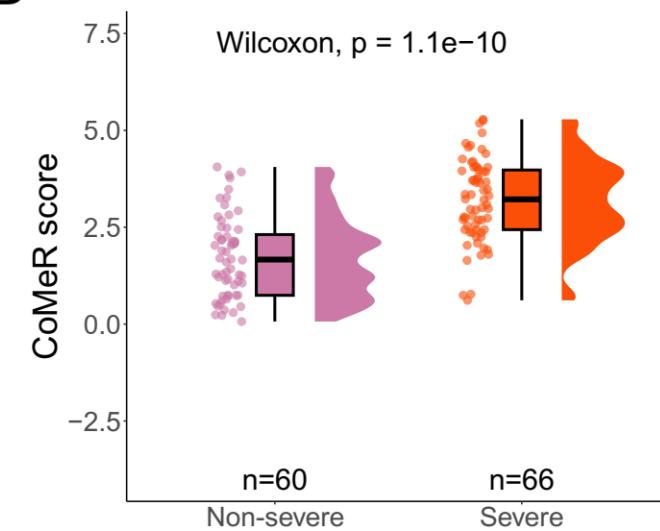
CoMeR score built from the 3 important probes



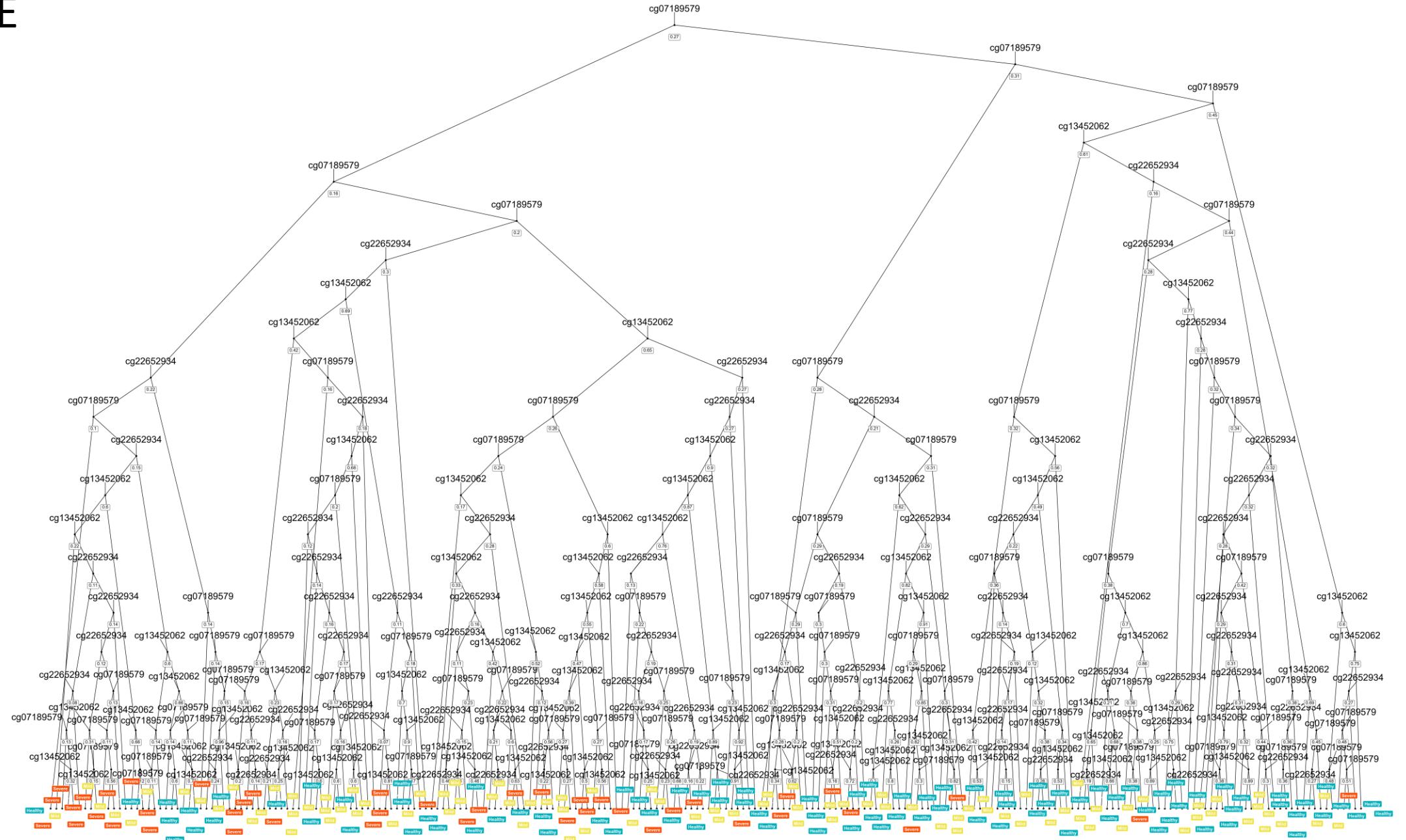
DMP	CHR	START	END	DIFF	MEAN DELTA (Severe/non.Severe)	GENE
cg13452062	chr1	79088559	79088559	0.0096	-0.223	IFI44L
cg07189579	chr8	134258444	134258444	0.0043	-0.109	NDRG1
cg22652934	chr21	36180035	36180035	0.0046	-0.088	RUNX1

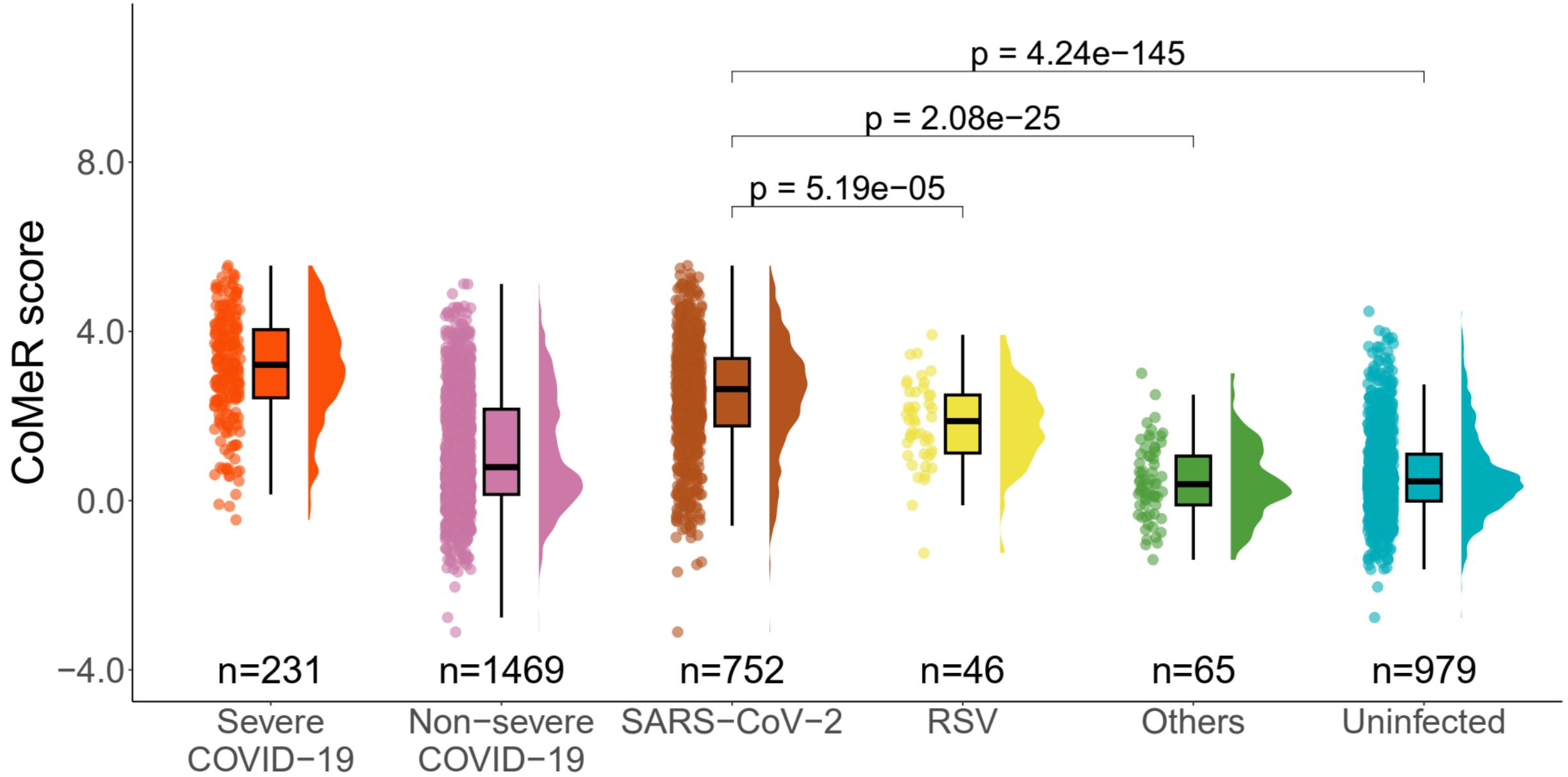
$$\text{CoMeR score} = 6.9642 - 6.3302 \times \beta_1 - 3.2855 \times \beta_2 + 6.7090 \times \beta_3$$

Where β_1 , β_2 , and β_3 are beta value of cg22652934, cg13452062, and cg07189579, respectively

A**B****C****D**

E





Polygenic Risk Score for Admixed Americans were predictive for COVID-19 severity



AUC = 0.54

(adapted from preliminary results of Christopher.H)

10.1007/s10557-020-07105-7

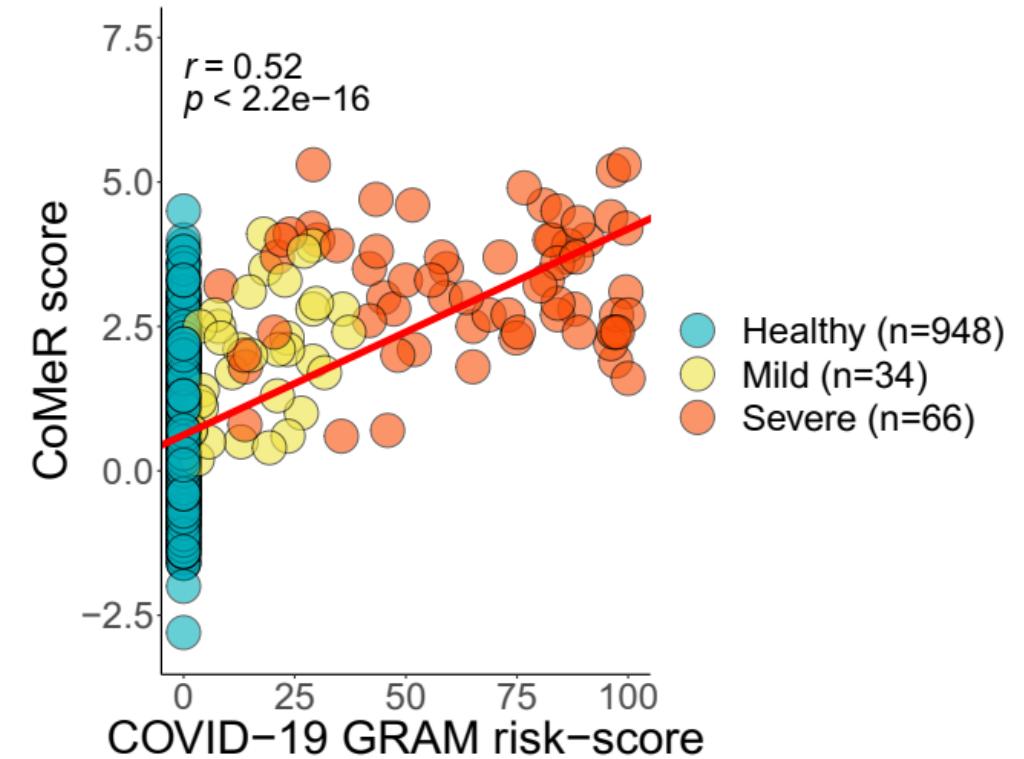
CoMER score is correlated with COVID-19 GRAM risk score

JAMA Internal Medicine | Original Investigation

Development and Validation of a Clinical Risk Score to Predict the Occurrence of Critical Illness in Hospitalized Patients With COVID-19

Wenhua Liang, MD; Hengrui Liang, MD; Limin Ou, MD; Binfeng Chen, MD; Ailan Chen, MD; Caichen Li, MD; Yimin Li, MD; Weijie Guan, MD; Ling Sang, MD; Jiatao Lu, MD; Yuanda Xu, MD; Guoqiang Chen, MD; Haiyan Guo, MD; Jun Guo, MD; Zisheng Chen, MD; Yi Zhao, MD; Shiyue Li, MD; Nuofu Zhang, MD; Nanshan Zhong, MD; Jianxing He, MD; for the China Medical Treatment Expert Group for COVID-19

=> AUC = 0.88



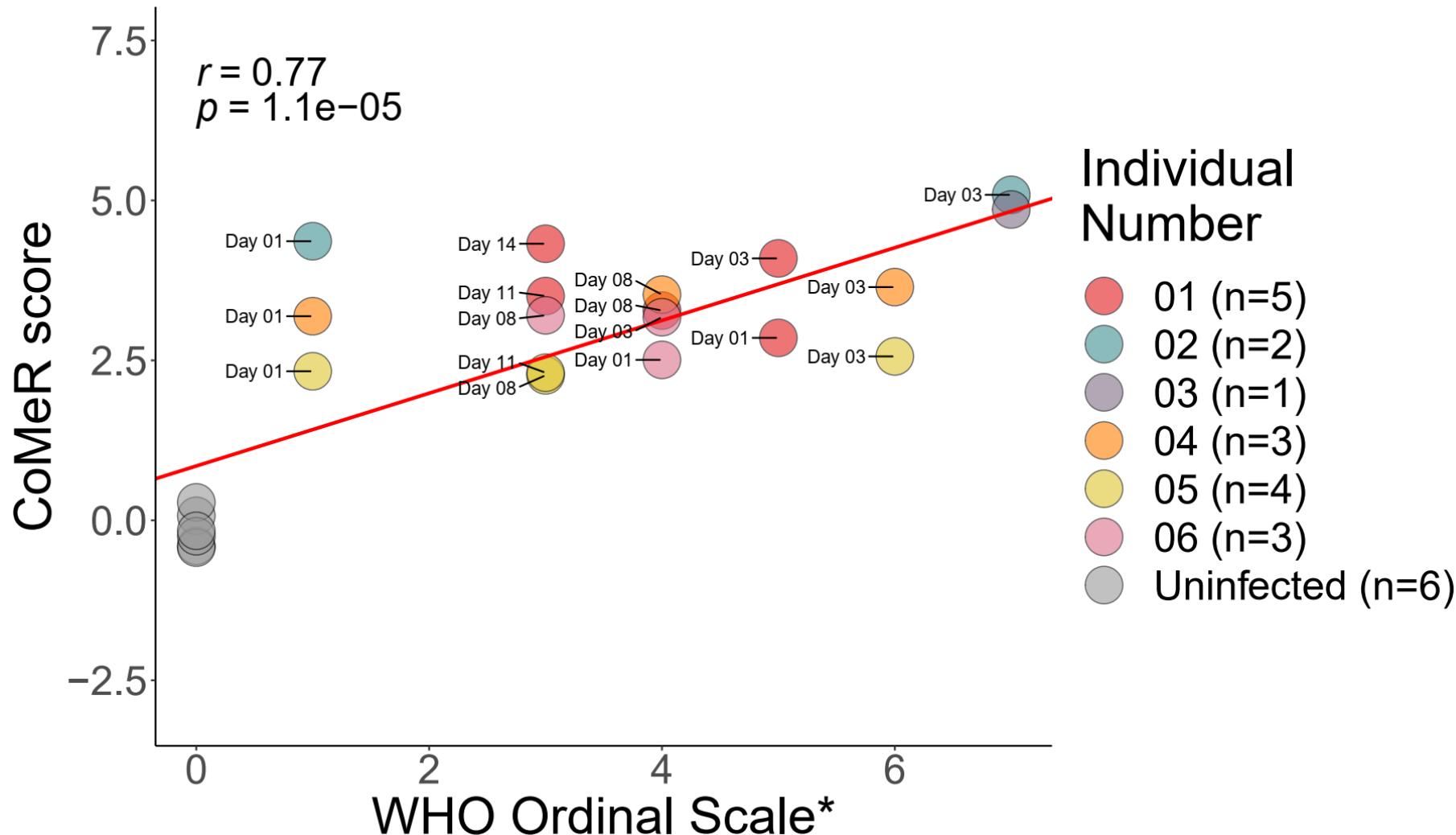
COVID-19 GRAM risk score

Based on **10 clinical factors**

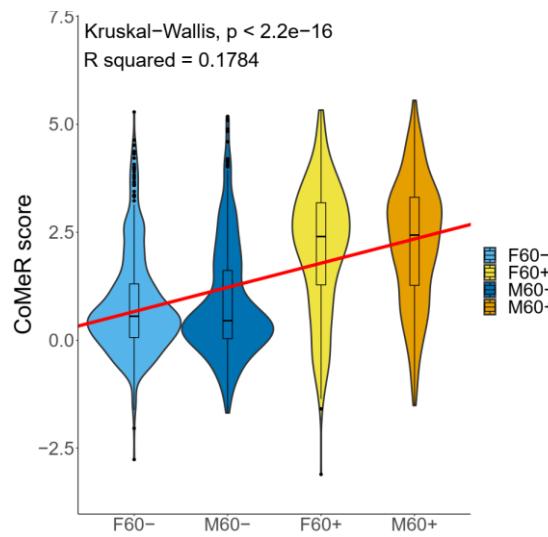
- chest radiographic abnormality
- Age
- Hemoptysis
- Dyspnea
- Unconsciousness
- number of comorbidities
- cancer history
- neutrophil-to-lymphocyte ratio
- lactate dehydrogenase
- direct bilirubin

=> AUC = 0.88

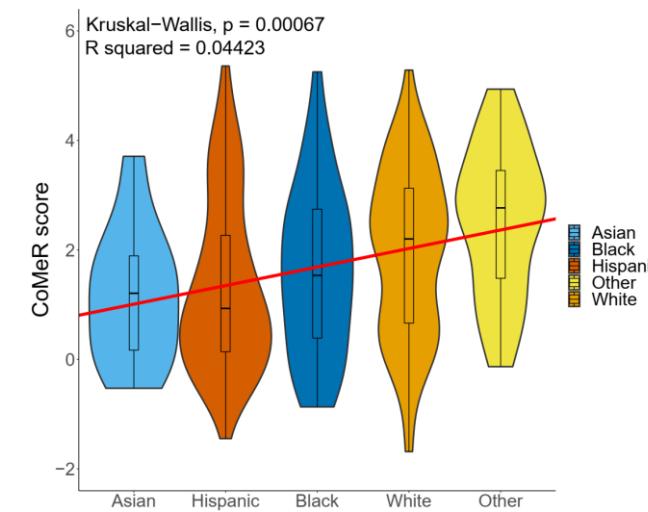
CoMER score is correlated with WHO Ordinal Scale



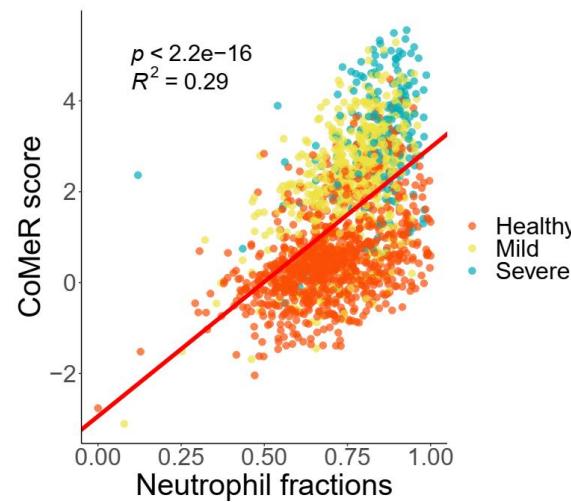
Age and Gender

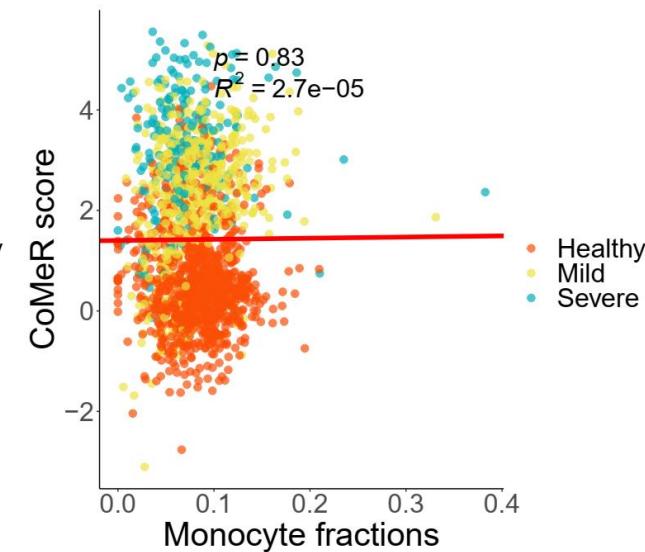
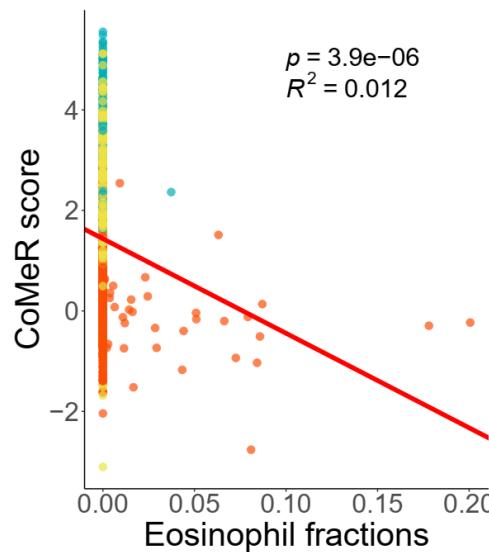
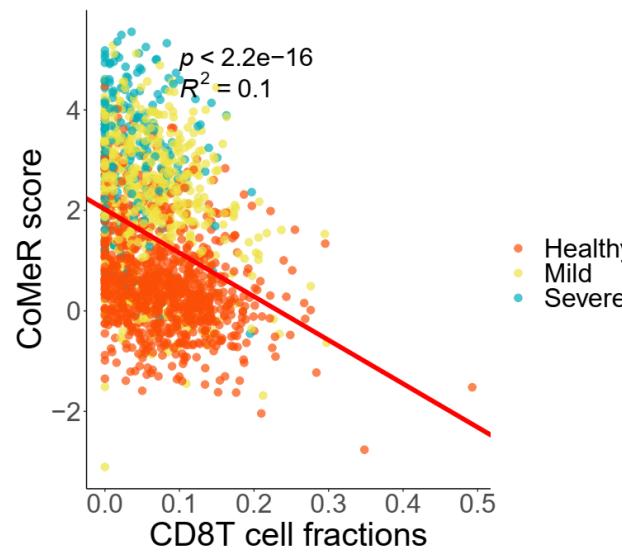
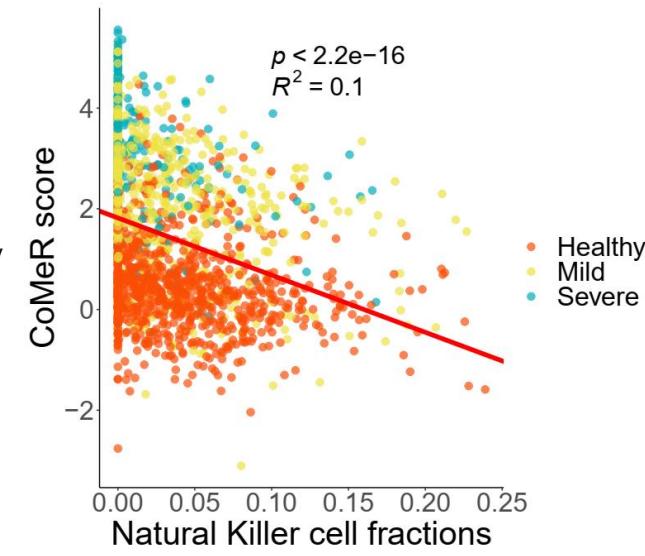
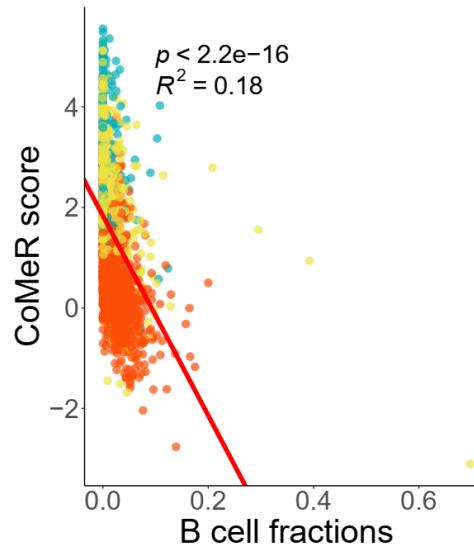
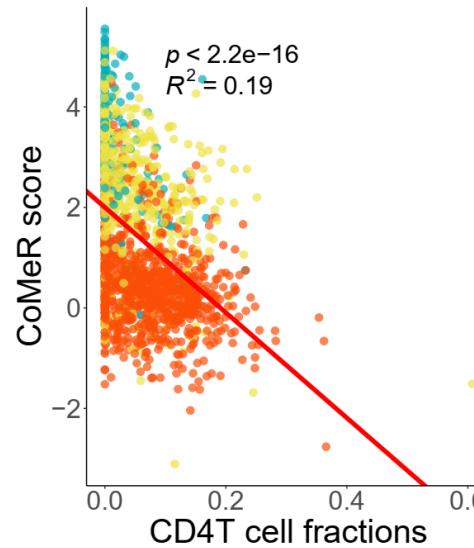


Race



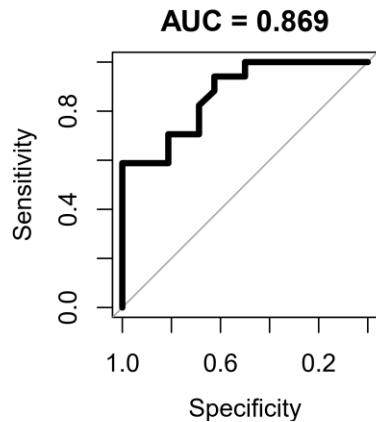
Neutrophil fractions



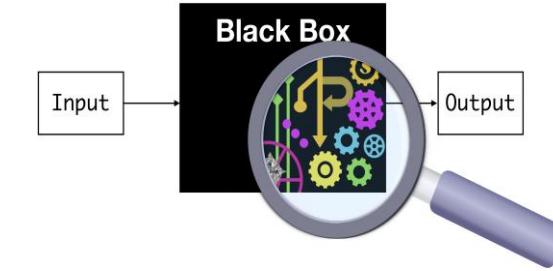
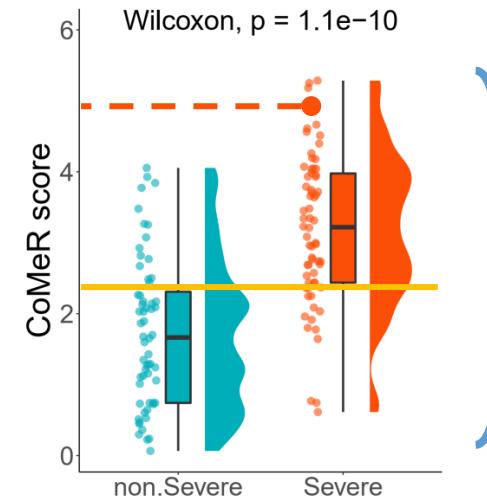
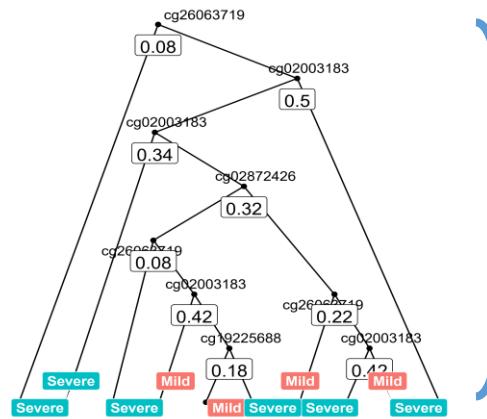
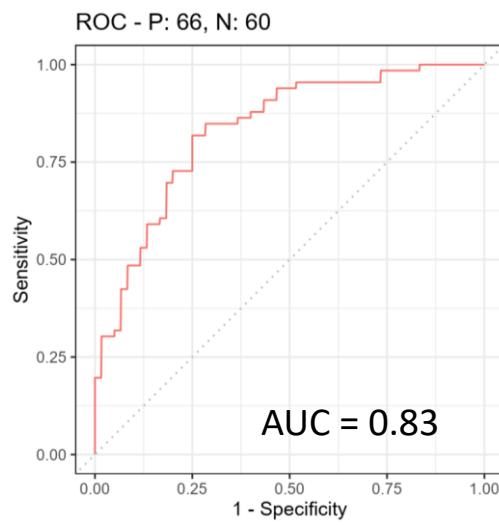


Advantage of CoMeR score over Machine learning classifiers

Random Forest



CoMeR score



Interpretable

ShinyApp: prediction of COVID-19 severity

Input parameters

Choose CSV File
 No file selected

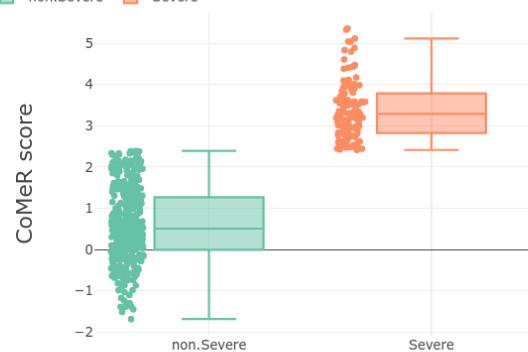
Search:

sample_ID	age	cg22652934	cg13452062	cg07189579
1 200863670076_R04C01	51	0.237827168635887	0.739874583385841	0.369289227438883
2 200863670076_R08C01	61	0.263254432354737	0.631592289582954	0.394676327863369
3 200863670077_R01C01	20	0.209298256450936	0.801910512040244	0.409720338247587
4 200863670088_R01C01	18	0.284337908261084	0.809006727379782	0.401993146463926
5 200863670088_R02C01	40	0.25646692805369	0.278072330577738	0.460221821422716
6 200863670090_R01C01	31	0.306489729140156	0.772366278710293	0.38177233349211
7 200863670090_R06C01	36	0.298833937300981	0.767507733951025	0.278099420932539
8 200863770046_R05C01	30	0.247927854622204	0.733383474449144	0.42003846331608
9 200863770070_R02C01	37	0.251751164663651	0.771392661844468	0.369661066800408
10 200863770070_R05C01	40	0.295995702773747	0.636170171290248	0.486332206371918

Showing 1 to 10 of 471 entries

Previous 1 2 3 4 5 ... 48 Next

Search:



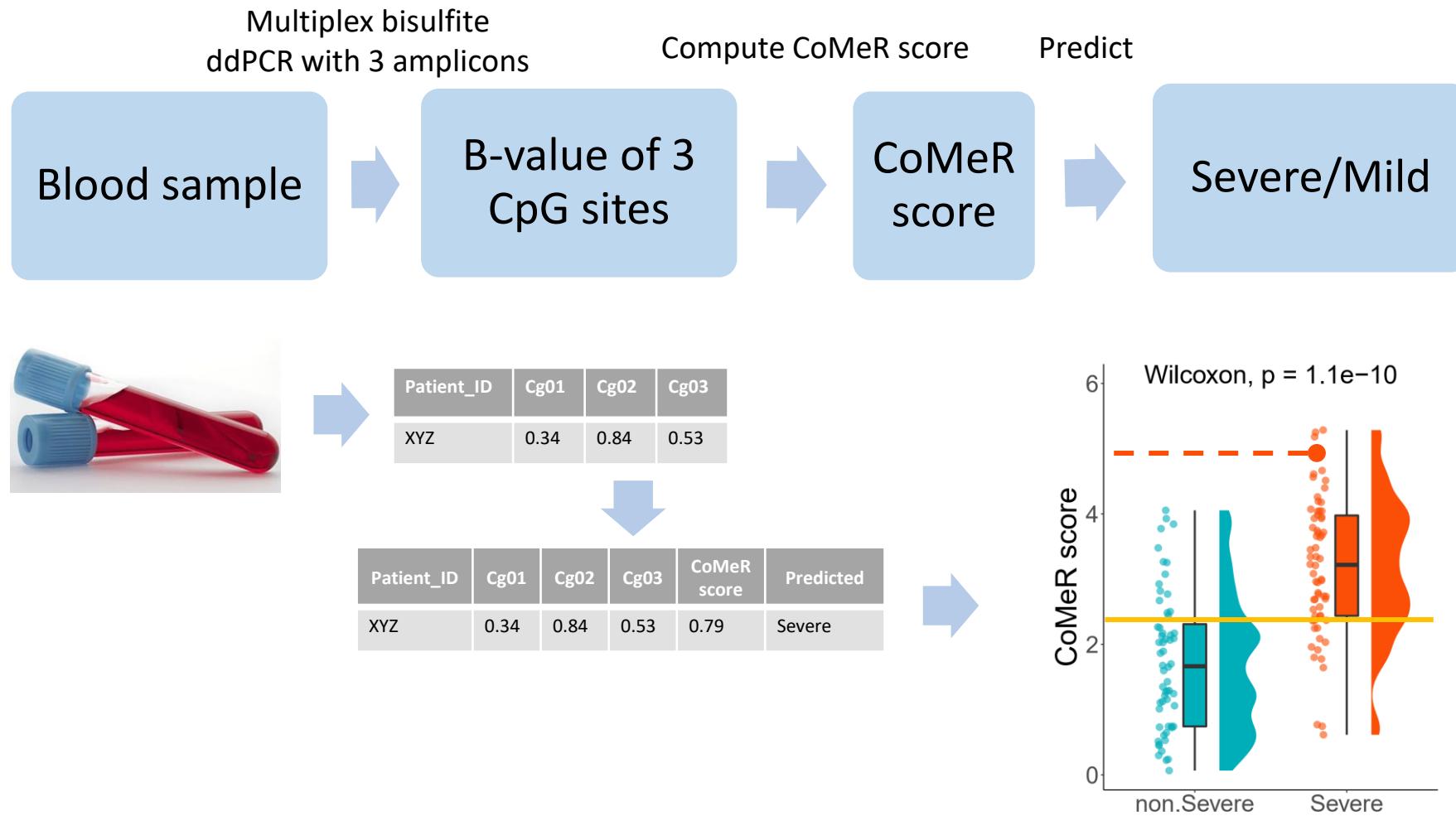
Scores in details and prediction

sample_ID	age	cg22652934	cg13452062	cg07189579	MRS_standard	predictive_status_standar
1 200863670076_R04C01	51	0.237827168635887	0.739874583385841	0.369289227438883	0.550308667339717	non.Severe
2 200863670076_R08C01	61	0.263254432354737	0.631592289582954	0.394676327863369	0.574790961195896	non.Severe
3 200863670077_R01C01	20	0.209298256450936	0.801910512040244	0.409720338247587	0.255832410432653	non.Severe
4 200863670088_R01C01	18	0.284337908261084	0.809006727379782	0.401993146463926	-0.190658201906353	non.Severe
5 200863670088_R02C01	40	0.25646692805369	0.278072330577738	0.460221821422716	1.33951128002769	non.Severe
6 200863670090_R01C01	31	0.306489729140156	0.772366278710293	0.38177233349211	-0.0748407643231124	non.Severe
7 200863670090_R06C01	36	0.298833937300981	0.767507733951025	0.278099420932539	0.685121525821732	non.Severe

Showing 1 to 10 of 471 entries

Previous 1 2 3 4 5 ... 48 Next

Future direction: CoMeR score for diagnosis of COVID-19 patient severity in clinic



Published: 14 March 2018

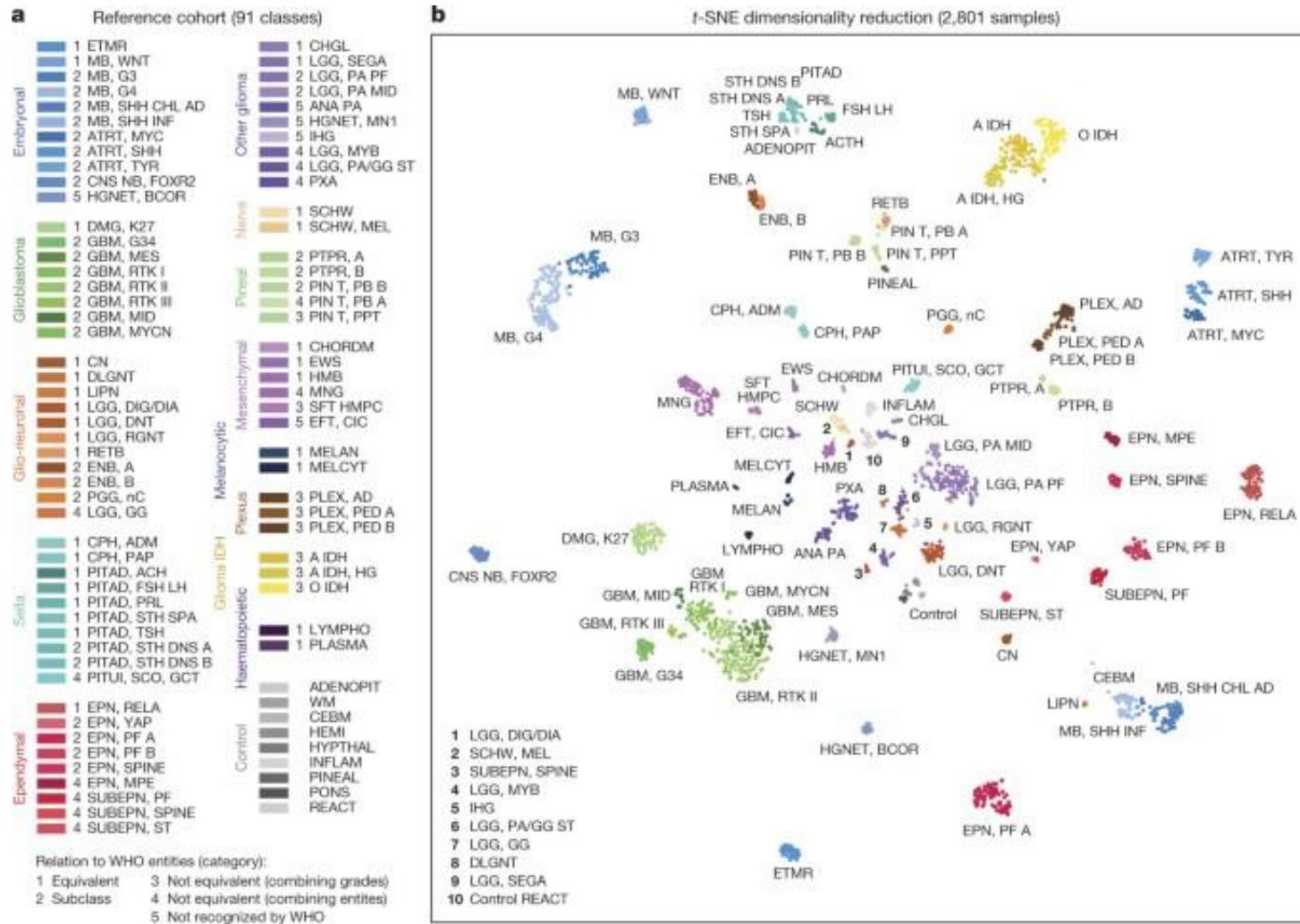
DNA methylation-based classification of central nervous system tumours

[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

[Nature](#) **555**, 469–474 (2018) | [Cite this article](#)

79k Accesses | **1309** Citations | **506** Altmetric | [Metrics](#)

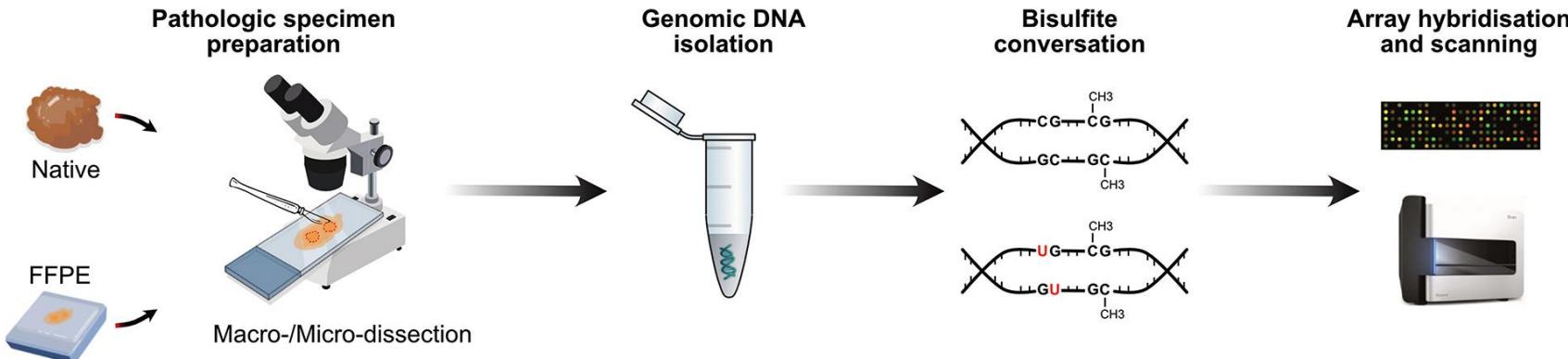
Other clinical application of DNA methylation in brain tumors



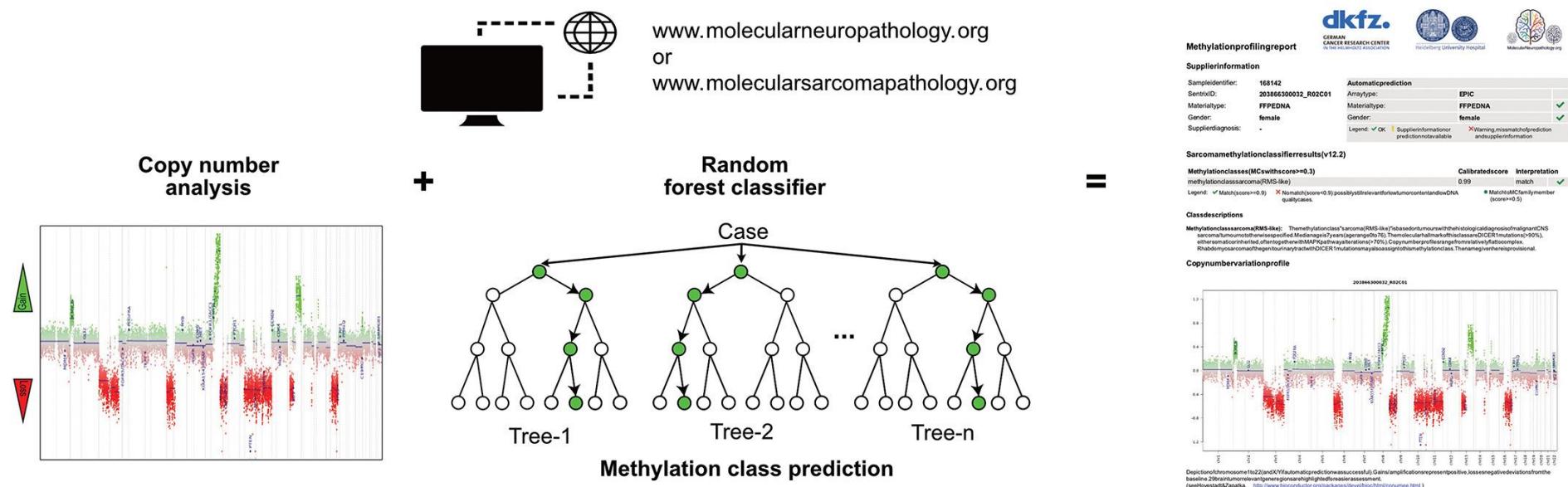
Methylation classifiers: Brain tumors, sarcomas, and what's next

- Koelsche - 2022 - Genes, Chromosomes and Cancer - Wiley Online Library

Data generation



Data visualization and classification

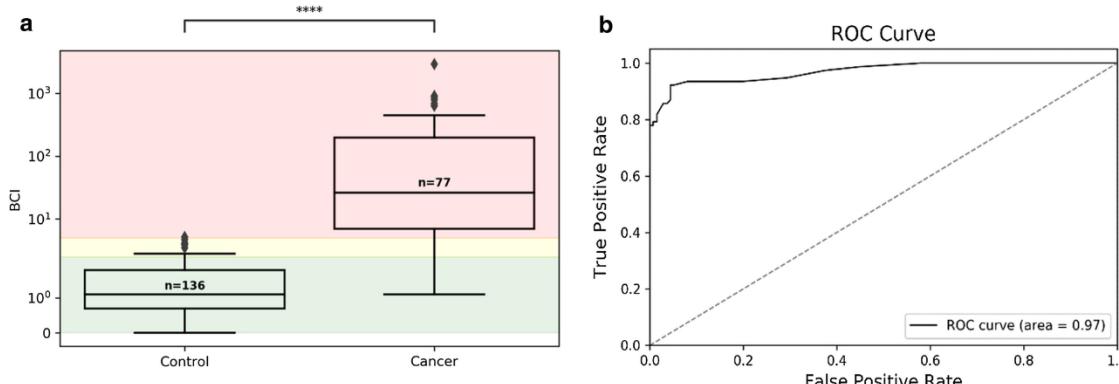


Clinical evaluation of Bladder CARE, a new epigenetic test for bladder cancer detection in urine samples

Paolo Piatti, Yap Ching Chew, Michiko Suwoto, Taikun Yamada, Benjamin Jara, Xi-Yu Jia, Wei Guo, Saum Ghodoussipour, Siamak Daneshmand, Hamed Ahmadi, Jeffrey Rice, Jeffrey Bhasin, Faith Holloway, Yvonne Tsai, Yoshitomo Chihara & Gangning Liang 

Clinical Epigenetics 13, Article number: 84 (2021) | [Cite this article](#)

2870 Accesses | 6 Citations | [Metrics](#)



- Collection bottle
- Urine stabilization agent
- Instructions
- Pre-paid return label

Doctor orders Bladder CARE
Test on patient's behalf



Bladder CARE Urine Collection Kit is delivered at patient's home address



At-home urine collection and stabilization



Shipment to Pangea Laboratory with standard shipping (room temperature) within 1 month from the date of sample collection



Sample received at Pangea Laboratory (CLIA and CAP)



- Sample de-identification
- Urine DNA purification and quantification
- Analysis of at least 5 ng of urine DNA with Bladder CARE Test



Generation and delivery of Patient Report to the doctor



NEGATIVE

HIGH-RISK

POSITIVE



For symptomatic patients:
Symptoms may be associated to other conditions (e.g., infection, kidney stones,..)

For surveillance patients:
No need to visit doctor office. Proceed with remote surveillance program

Patient requires immediate attention:
Confirmation of the presence of tumor with cystoscopy

THANK YOU FOR YOUR ATTENTION!

Please contact:

Email: luu.p.loi@googlemail.com

Zalo: 0901802182

For further information!