Copy number and methylation profiling of circulating cell free DNA for minimally invasive classification of high-grade lymphoma

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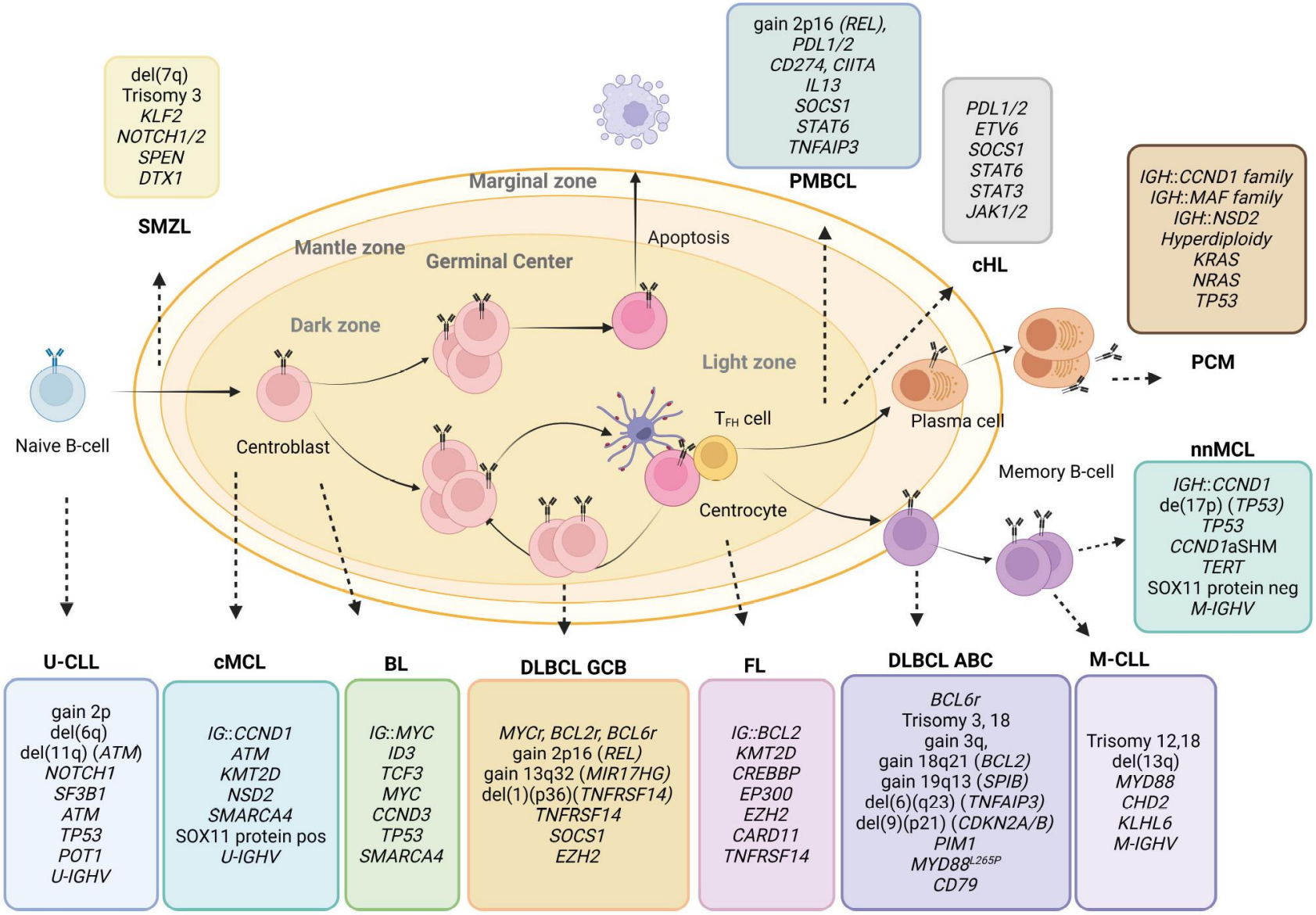
azdelta

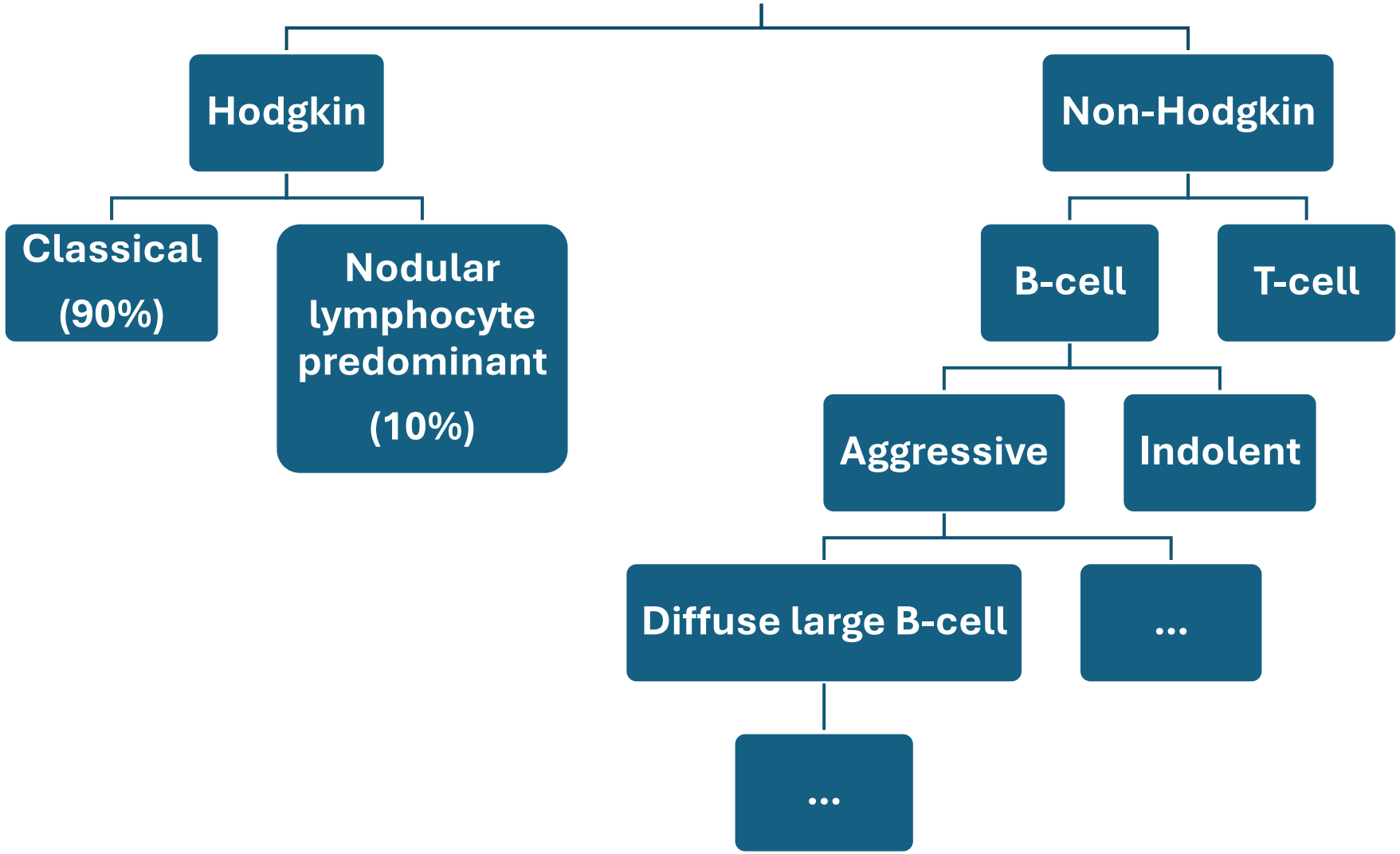
lymphoma classification



- prognostic
- therapeutic

C López, 2024

lymphoma classification



liquid biopsy-based research

blood collection / ”liquid biopsy” → plasma prep → cfDNA isolation → cfDNA library prep → cfDNA analysis

“cell-free DNA” (cfDNA)

copy number profiling

methylation profiling

ARTICLE

Shallow-depth sequencing of cell-free DNA for Hodgkin and diffuse large B-cell lymphoma (differential) diagnosis: a standardized approach with underappreciated potential

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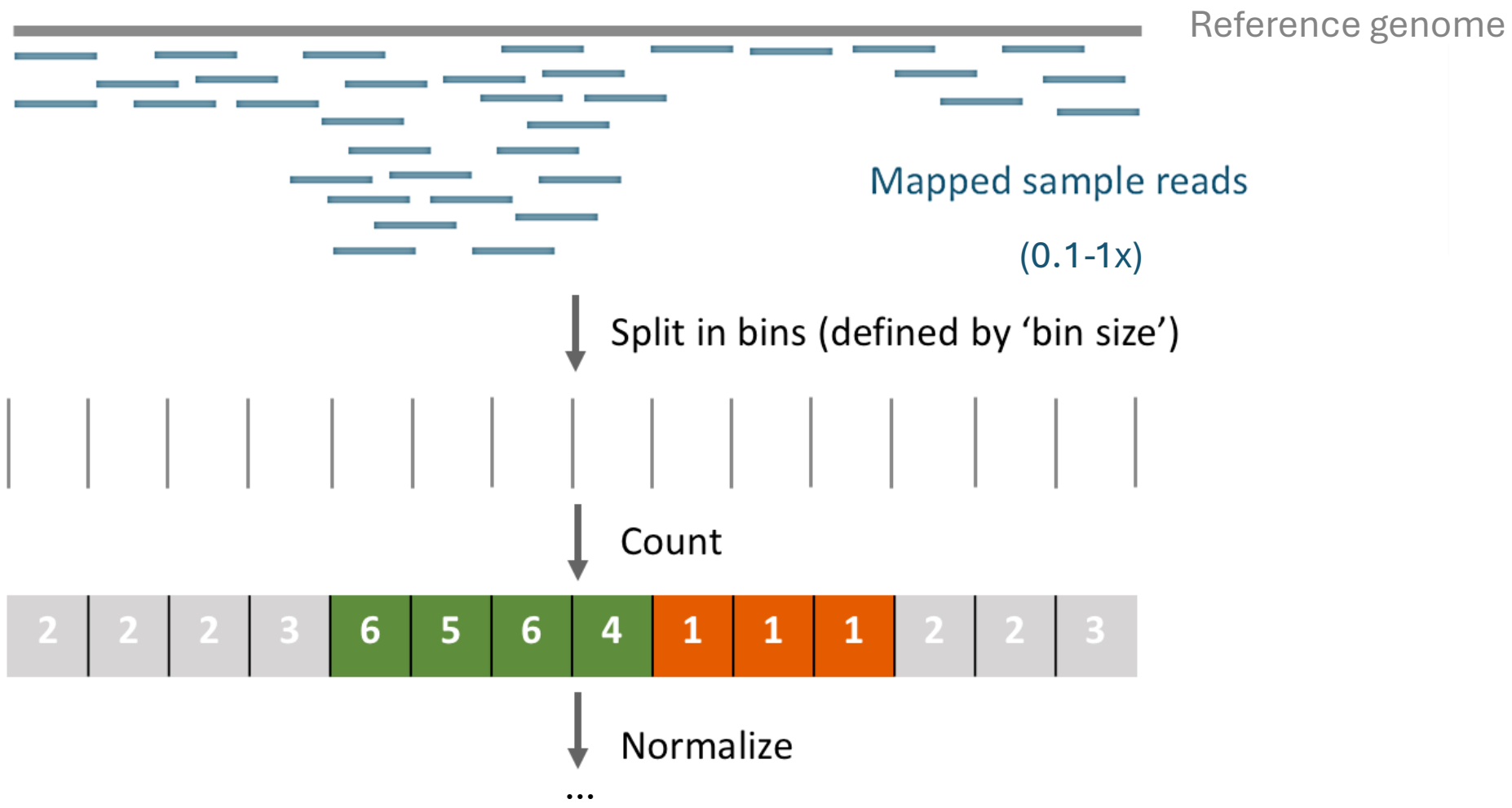
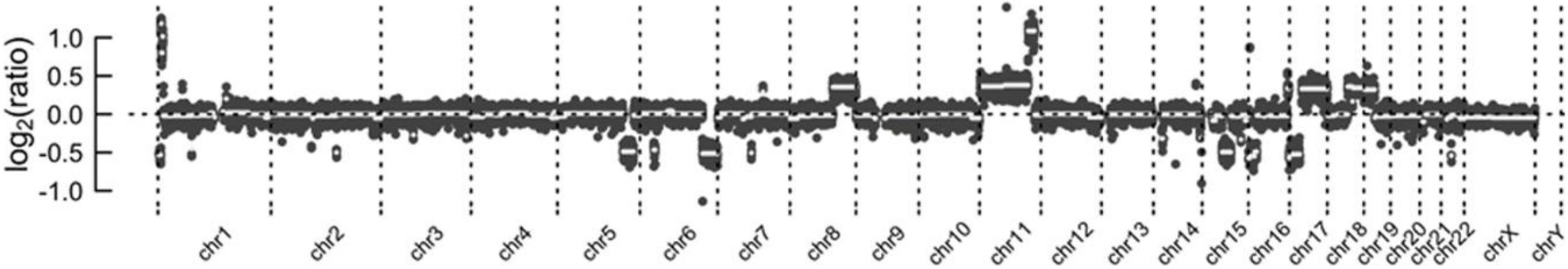
shallow whole-genome sequencing for copy number profiling



copy number profiling

WisecondorX

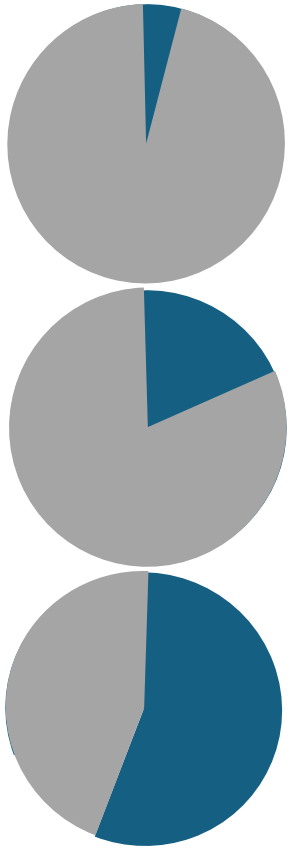


copy number profiling on cell-free DNA

< 4% ctDNA

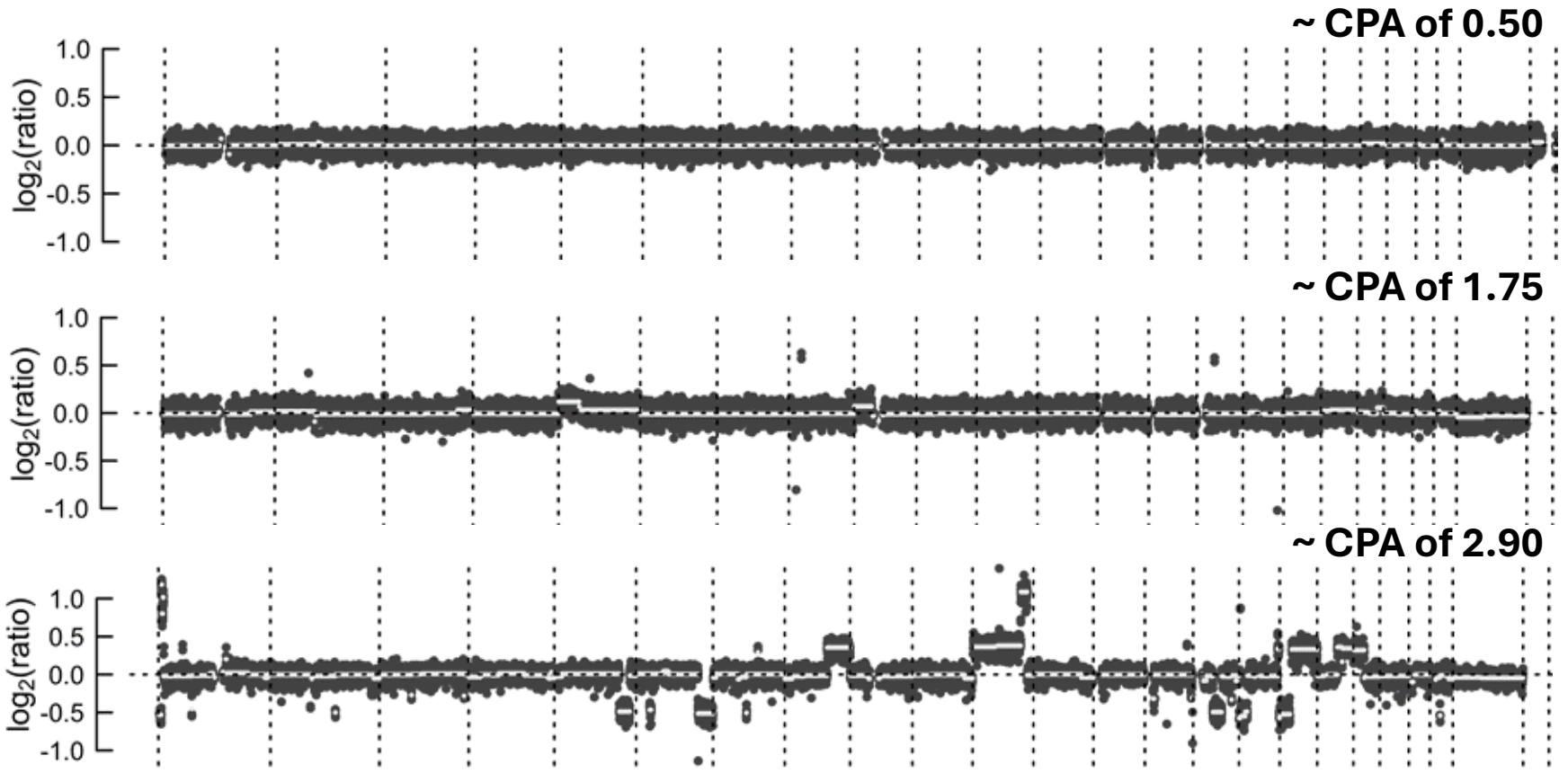
~ 18% ctDNA

~ 55% ctDNA



normal cfDNA

circulating tumor DNA (ctDNA)



a “**copy number profile abnormality (CPA) score**”

study cohort

123 patients
- 38 Hodgkin
- 85 non-Hodgkin (diffuse large B-cell)

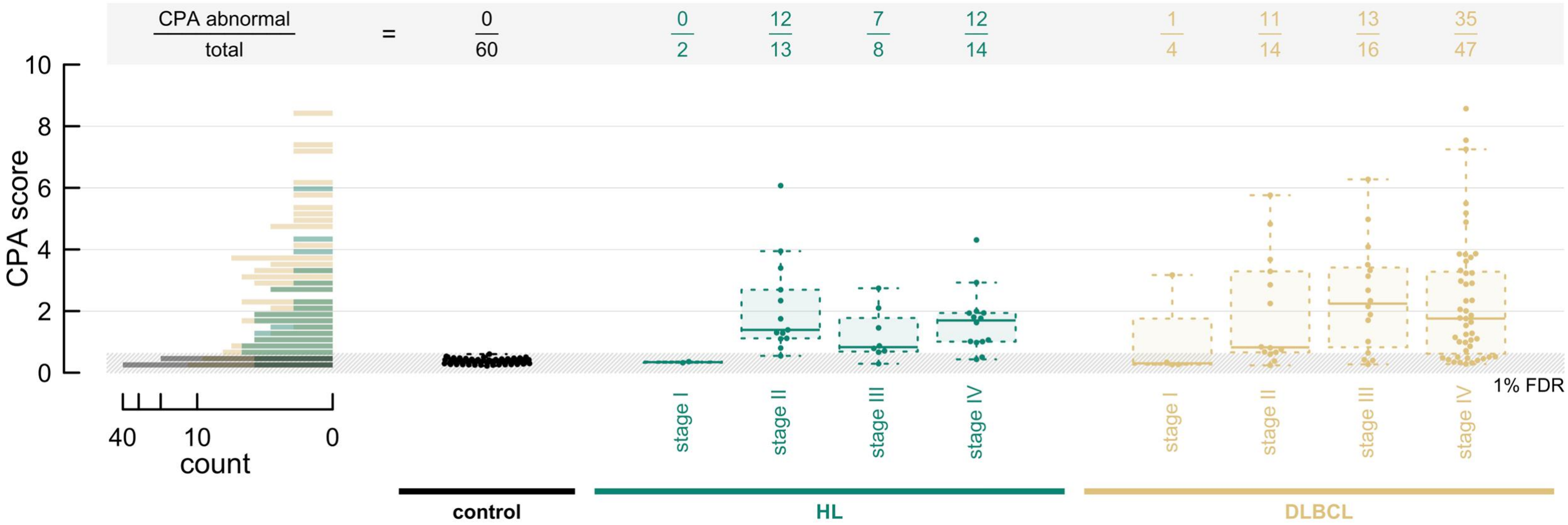
31 patients
- 9 Hodgkin
- 22 non-Hodgkin (diffuse large B-cell)

123 liquid biopsies

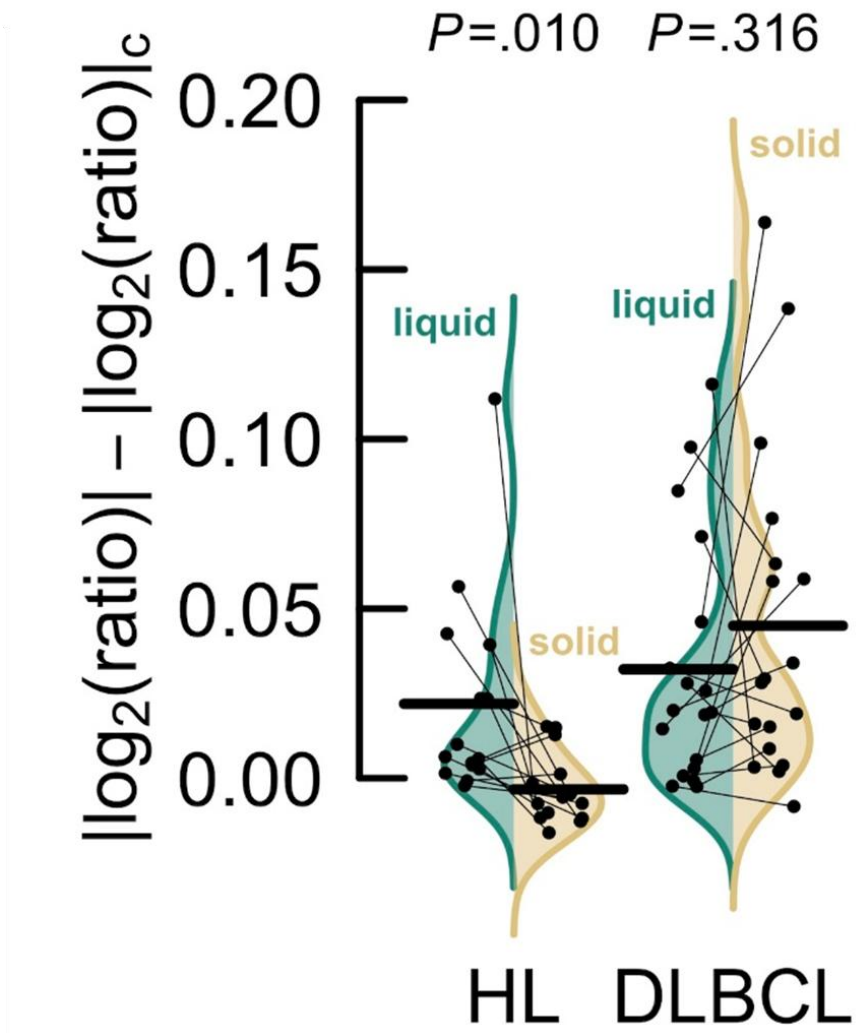
93 liquid biopsies

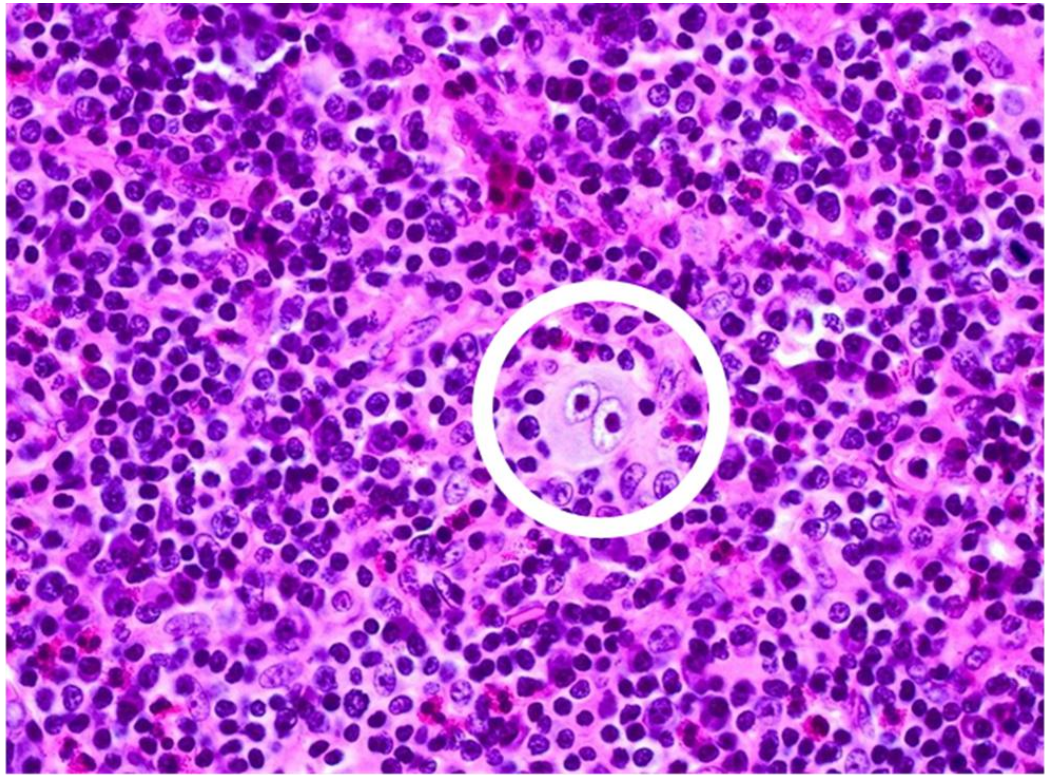
33 tissue biopsies
- 15 Hodgkin
- 18 diffuse large B-cell

sensitivity of 89% for classical Hodgin lymphoma and 74% for diffuse large B-cell lymphoma



liquid biopsy > solid biopsy for classical Hodgkin lymphoma





rare Reed-Sternberg-cells in background

Epstein-Barr Virus (EBV)-induced lymphoma

Latent infection with EBV

↓

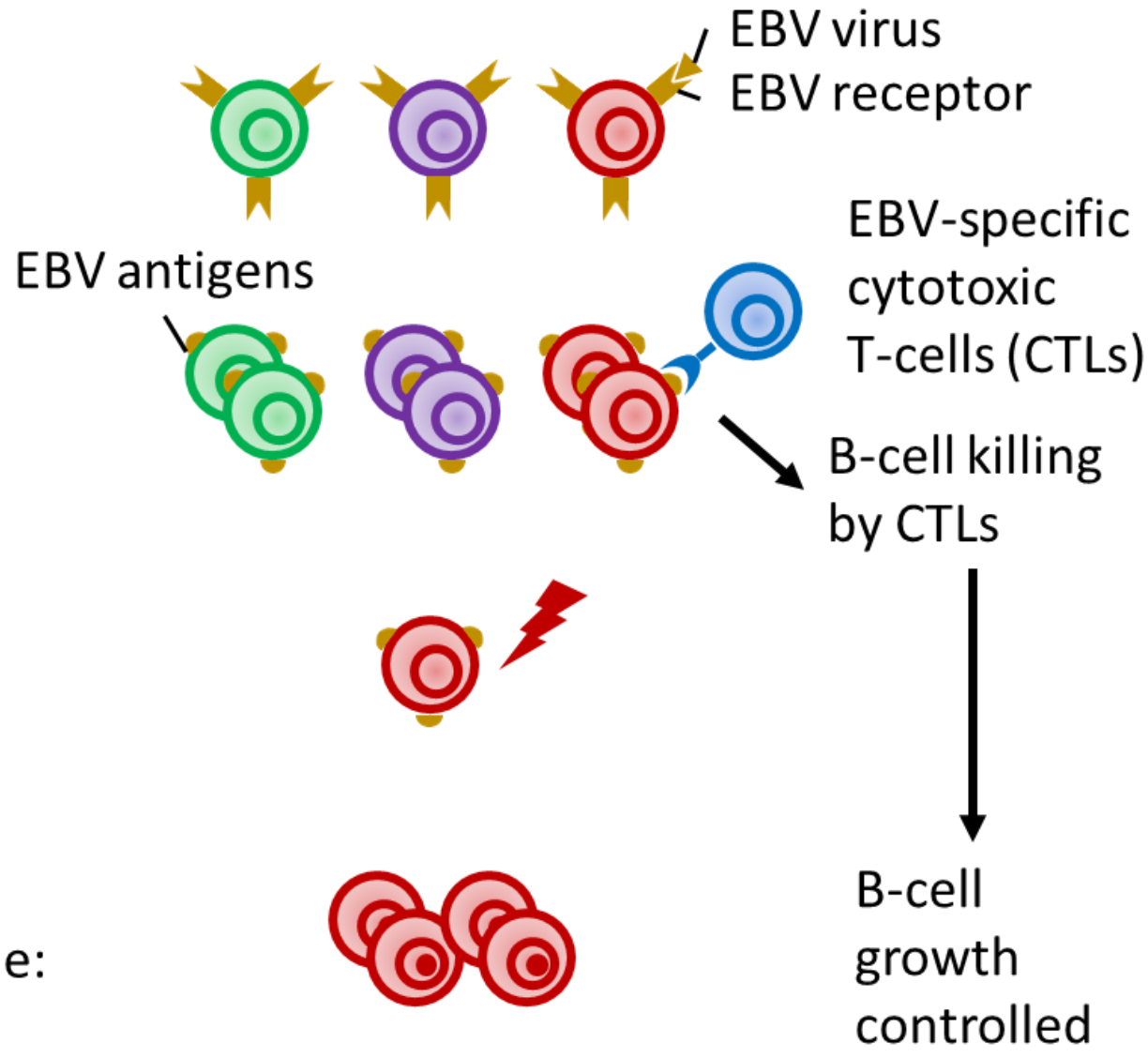
Polyclonal B-cell expansion

↓

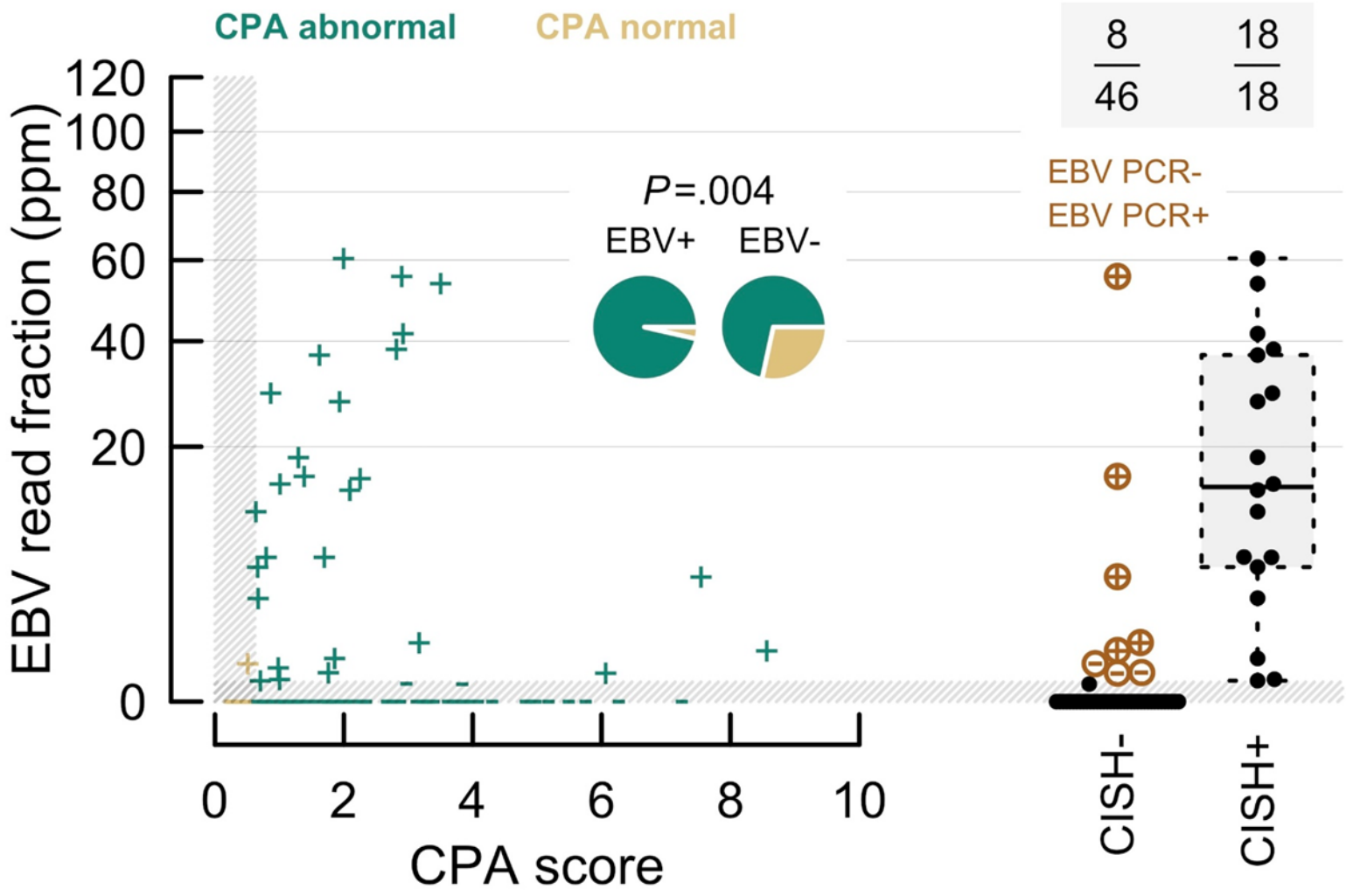
Genetic changes

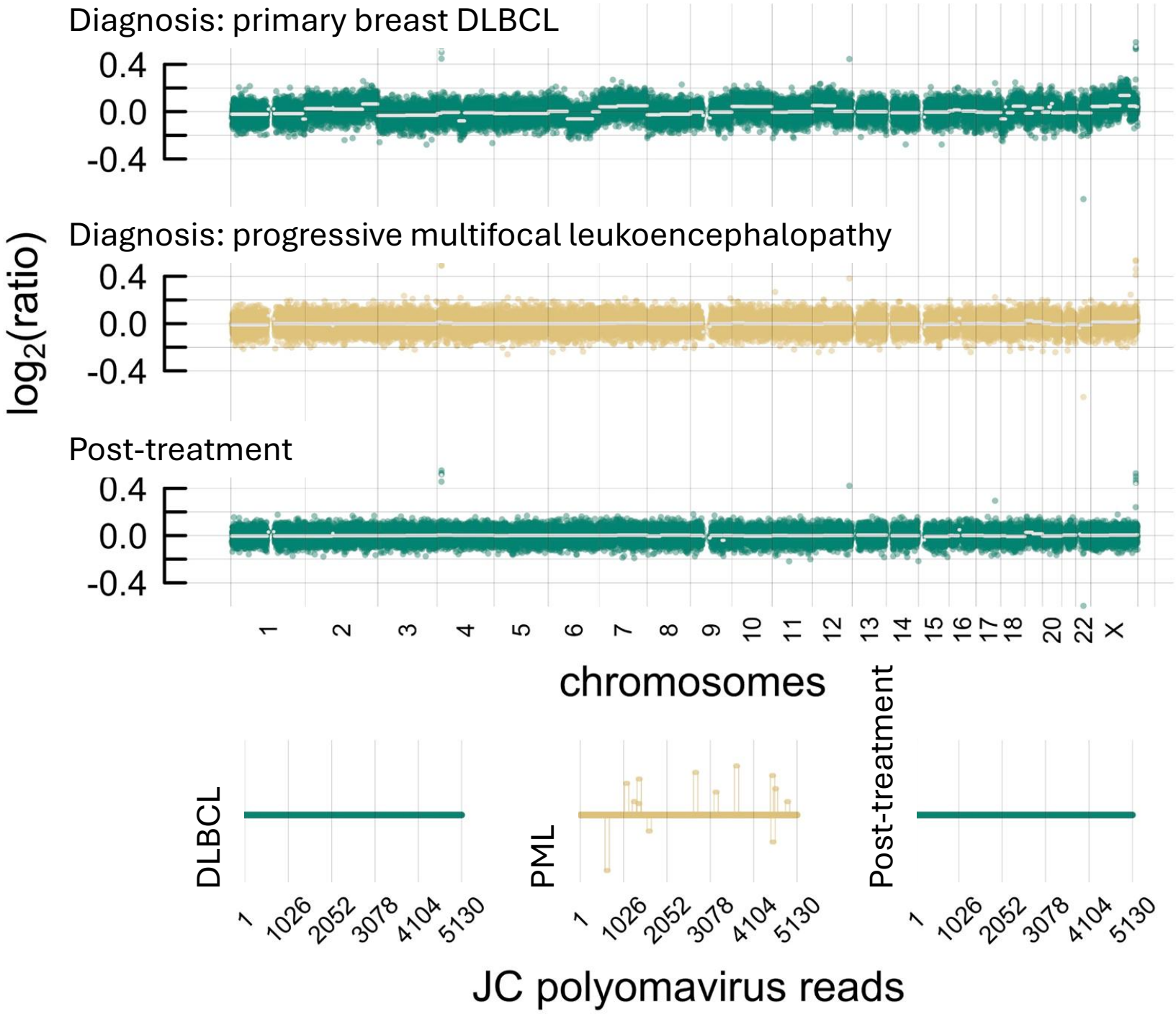
↓

Outgrowth of neoplastic clone: lymphoma

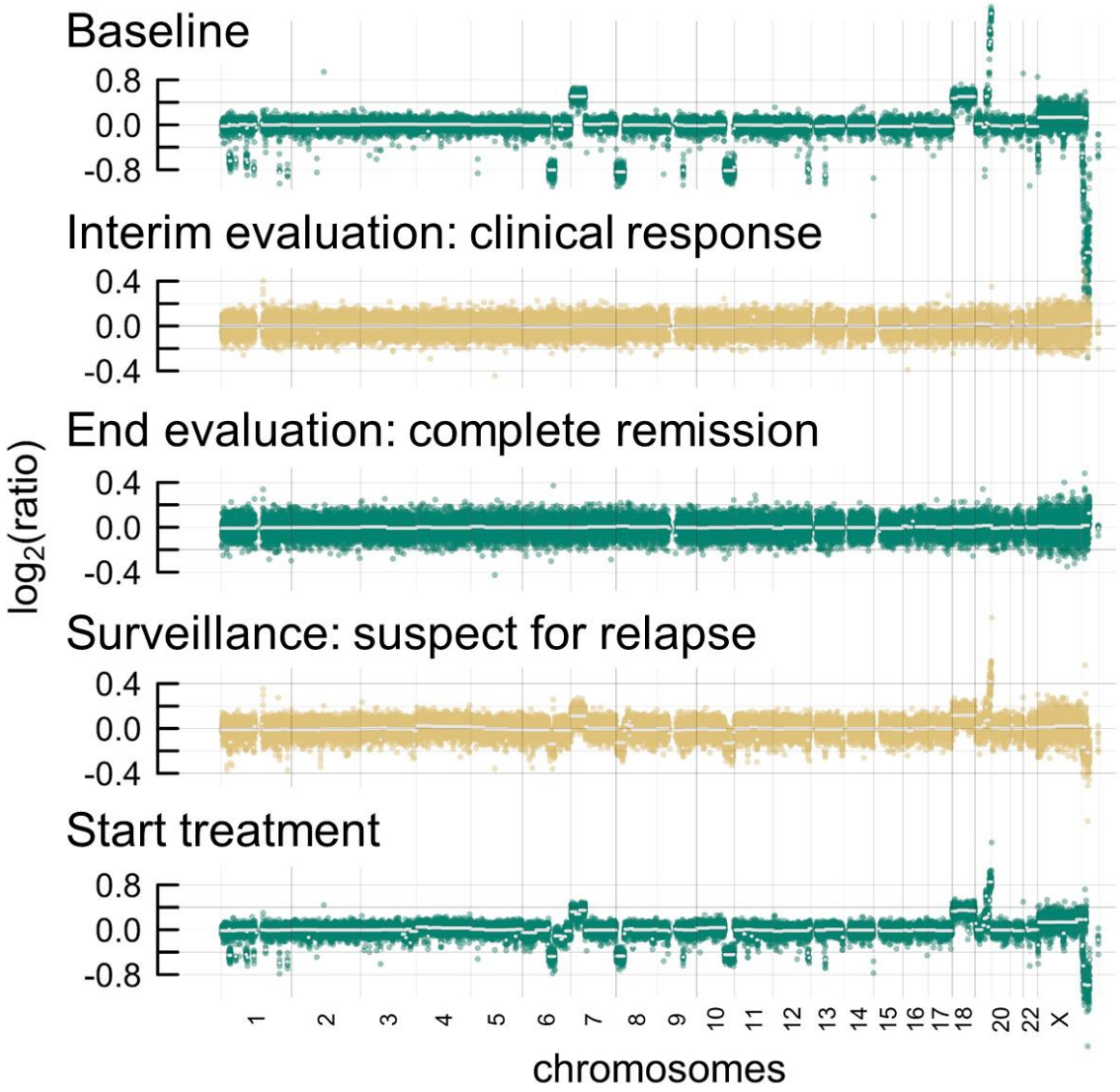


100% sensitivity for EBV detection

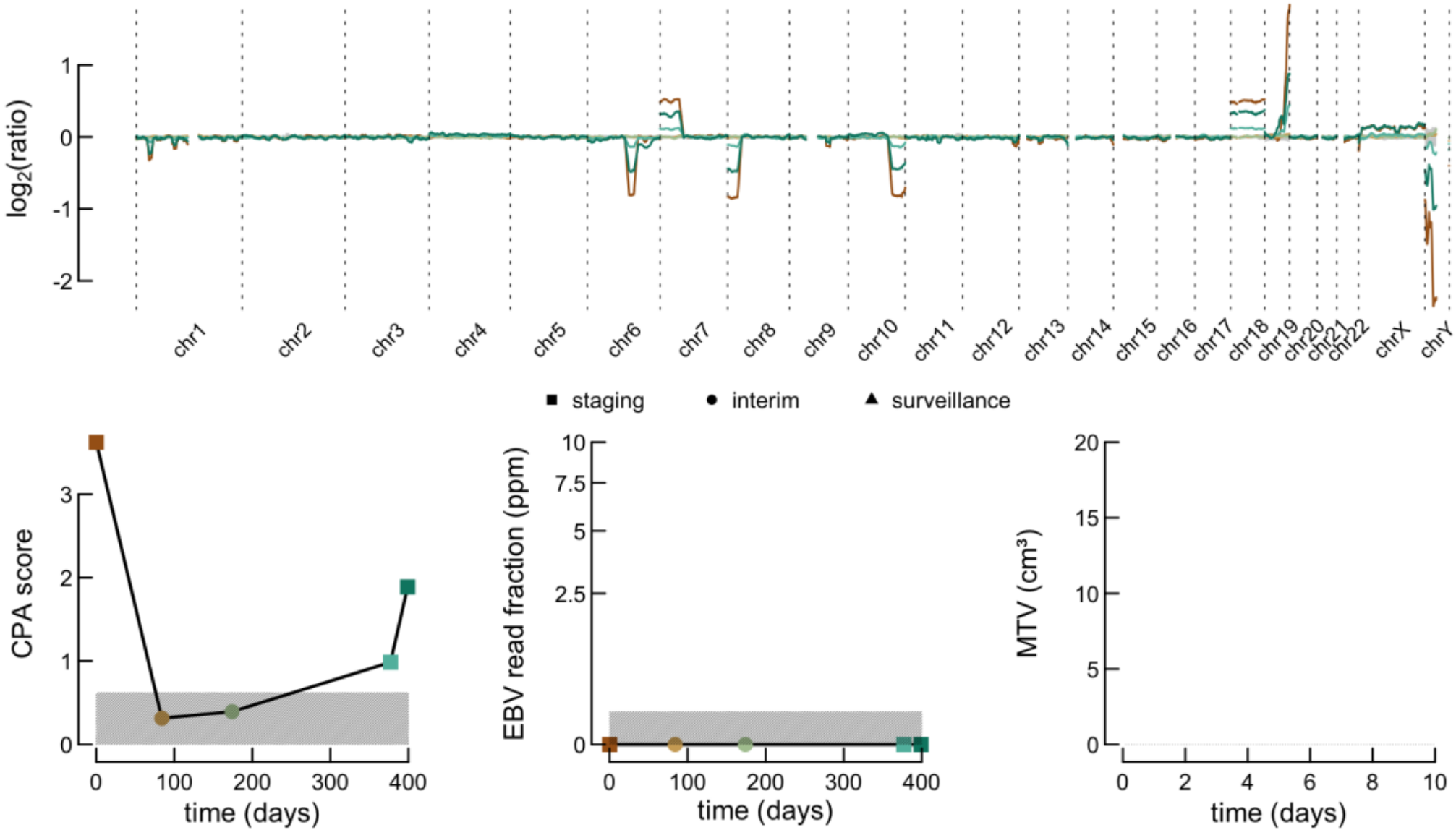



Diagnosis: primary breast DLBCL
0.4
0.0
-0.4
Diagnosis: progressive multifocal leukoencephalopathy
log2(ratio)
Post-treatment
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 20 22 X
chromosomes
DLBCL
PML
Post-treatment
1 1026 2052 3078 4104 5130
JC polyomavirus reads

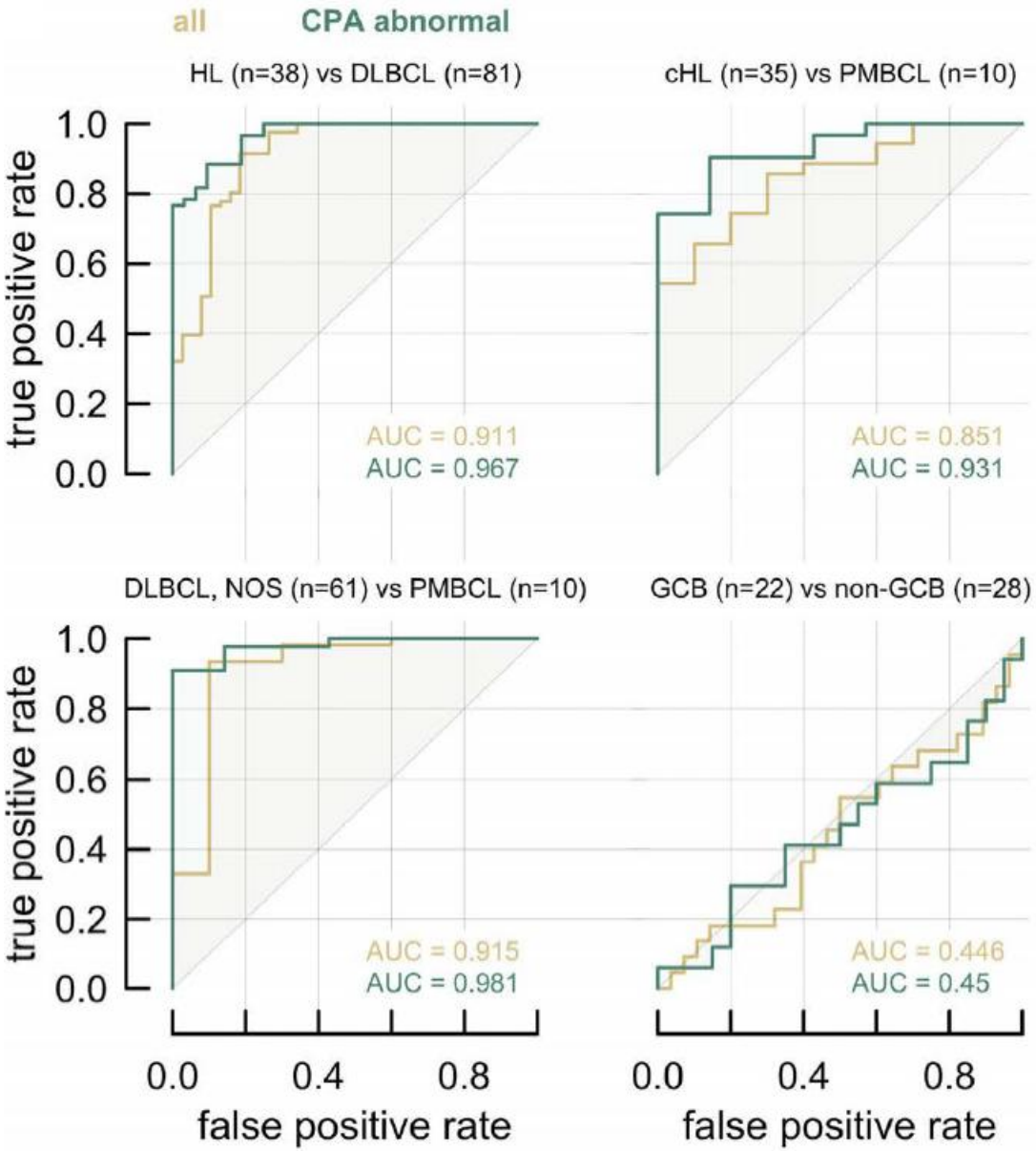
copy number profiles correlate with disease status



copy number profiles correlate with disease status



copy number profiles could aid histological classification



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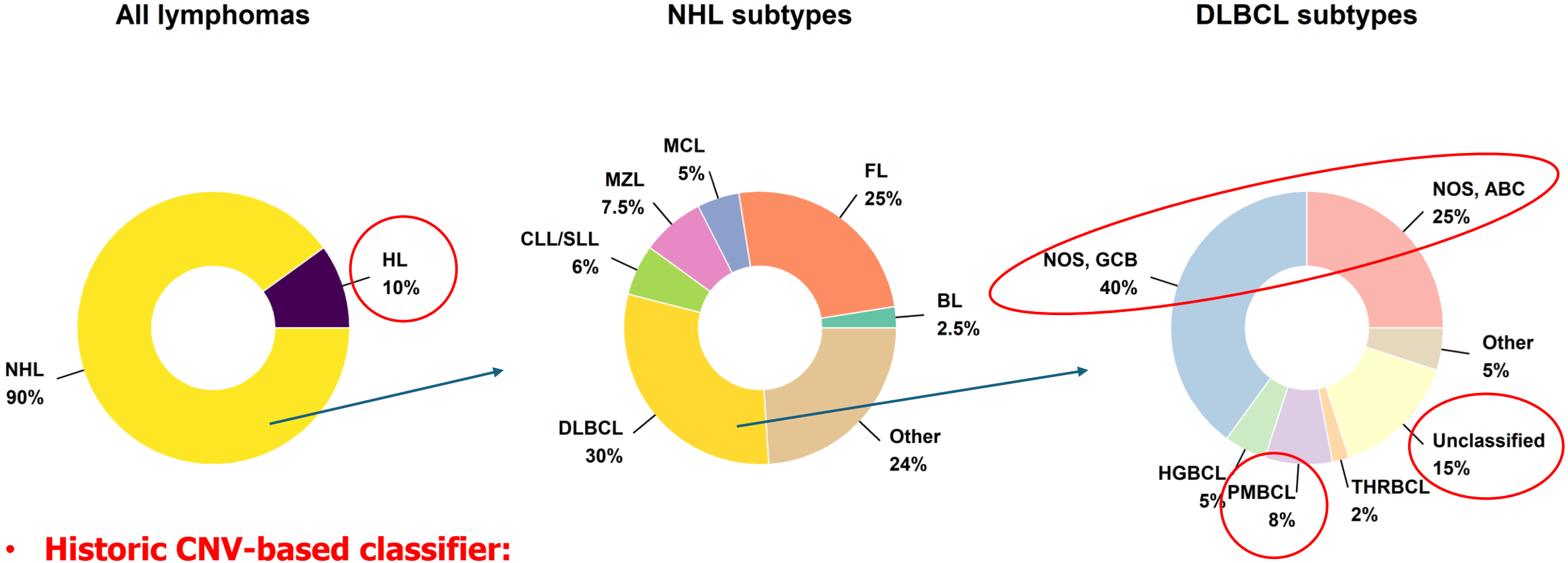
CRIG member

Doctoral fellow - Department of Pathology, Center for Medical Genetics (Faculty of Medicine and Health Sciences, UGent) & VIB Center for Medical Biotechnology (VIB-CMB)
Principal Investigators: Prof. Jo Van Dorpe (MD, PhD), Prof. Björn Menten (PhD) and Prof. Katleen De Preter (PhD)



- **Historic CNV-based classifier:**
  - "Capable" of classifying ~40% of major lymphoma cases (optimistic estimate)
  - DLBCL alone is highly heterogeneous with plenty of subtypes based on genetics and COO

- Are the subtypes not covered by the model relevant? **Yes!**
  - **GCB vs. ABC:** GCB generally better survival and may respond differently to targeted treatments
  - **DLBCL vs. FL:** FL is indolent but can transform into DLBCL, affecting treatment planning
  - **HGBCL vs. DLBCL, NOS:** HGBCL is more aggressive with poorer outcomes and often requires intensified chemotherapy

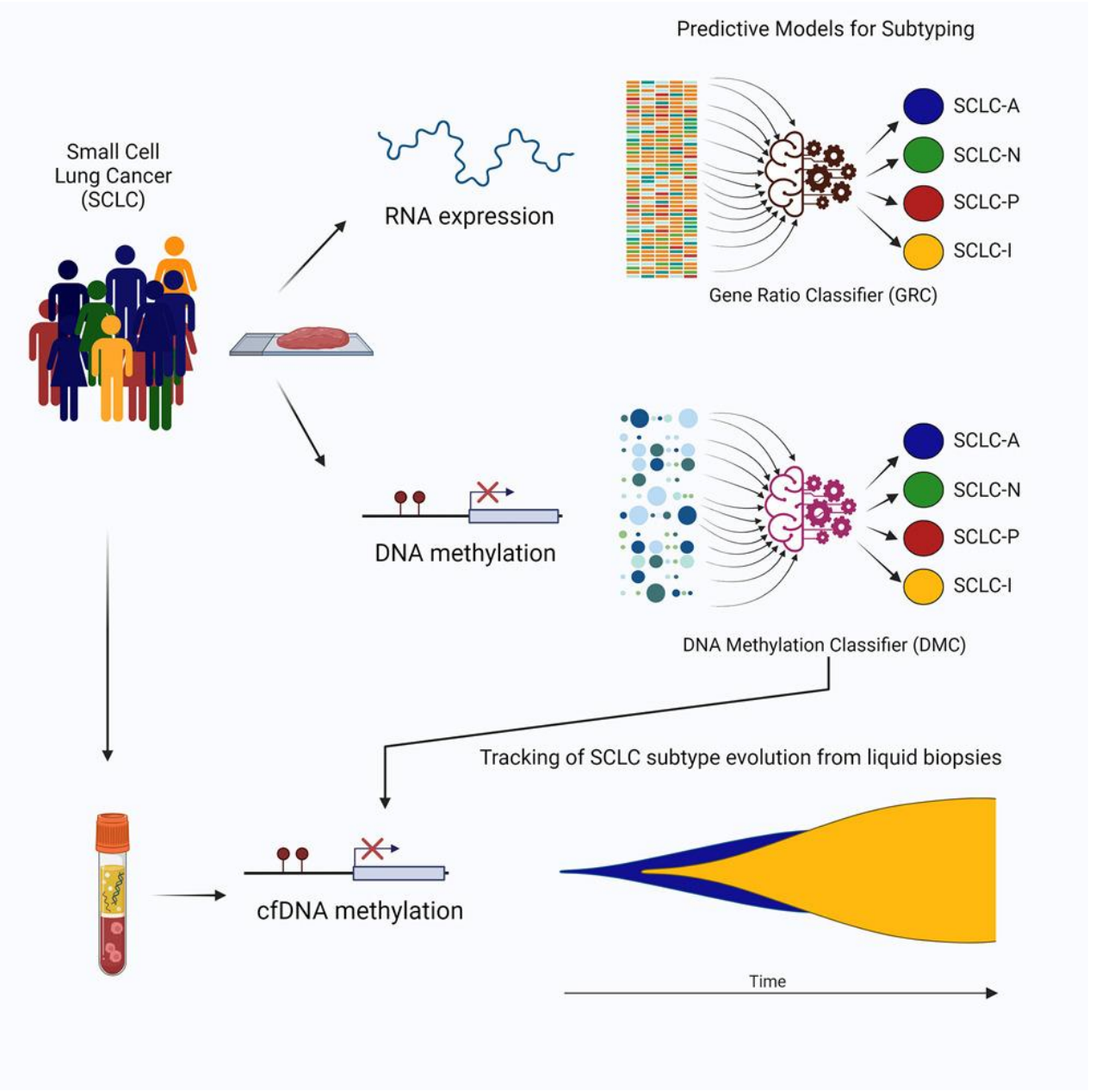
Methylation profiling > CNVs
(for accurate cancer subtyping)

1. **Cell identity & state**
   Methylation: cell of origin & differentiation
   CNVs: minimal insight into regulatory programs

2. **Stability**
   Methylation: signal remains stable in cfDNA
   CNVs: can be noisy or variable, subclonality

3. **Subtype specificity**
   Methylation: locus-specific
   CNVs: genome-wide instability

4. **Comprehensiveness**
   Methylation: CpGs + CNA
   CNVs: Only CNA



Clinical adoption + cfDNA → trade-off of cost, reproducibility, and handling fragmented input!

Platform	Type	Pro	cfDNA Limitation
WGBS	Genome-wide, bisulfite	Full genome coverage	High input, DNA loss
RRBS	CpG-enriched, bisulfite	Low cost, CpG focus	Fragmentation, poor enrichment
EM-seq	Enzymatic conversion	Preserves DNA, low input	Needs optimization
Illumina EPIC	Array, bisulfite	High throughput, robust	Limited sites, high input
Nanopore	Direct, long-read	Native methylation	Low accuracy, fragmented cfDNA

RRBS

Restriction enzyme digest

Size selection

Bisulfite conversion

RRBS

Restriction enzyme digest

Size selection

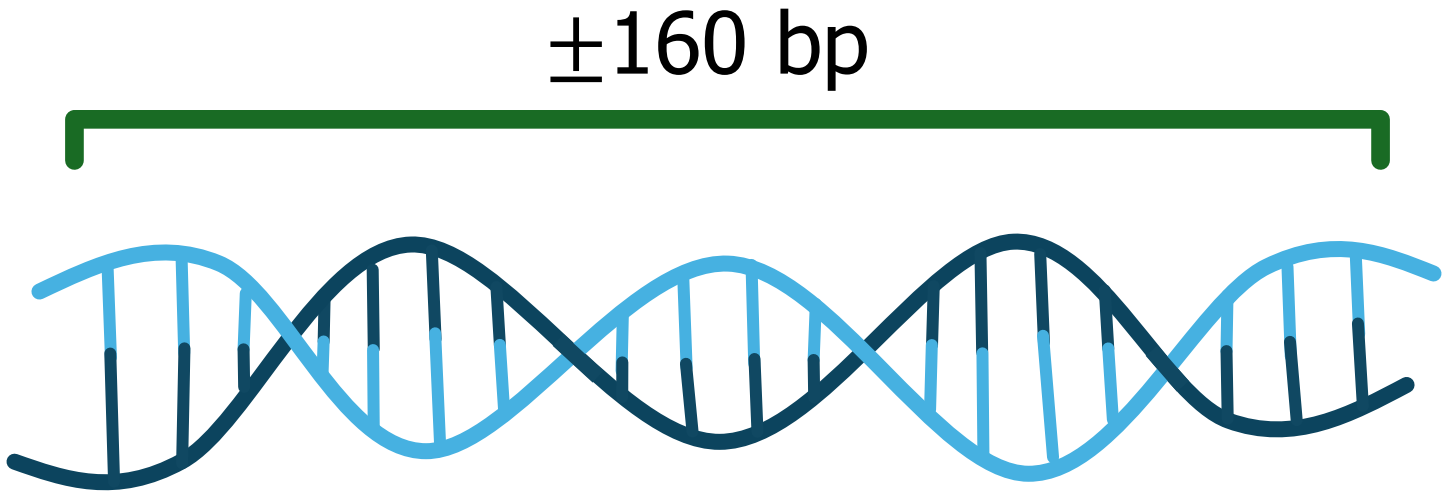
Bisulfite conversion

cfRRBS

Restriction enzyme digest

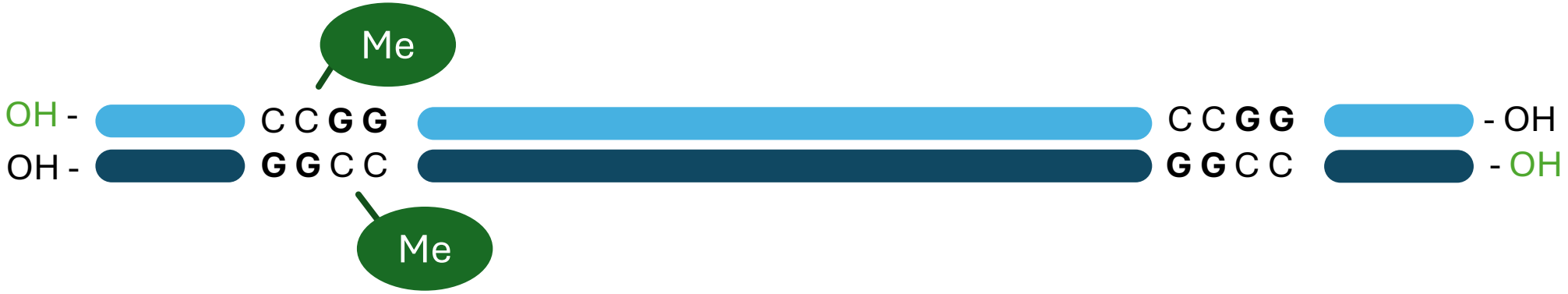
Size ction

Bisulfite conversion



Me
P -
C C G G
C C G G
- OH
OH -
G G C C
G G C C
- P
Me

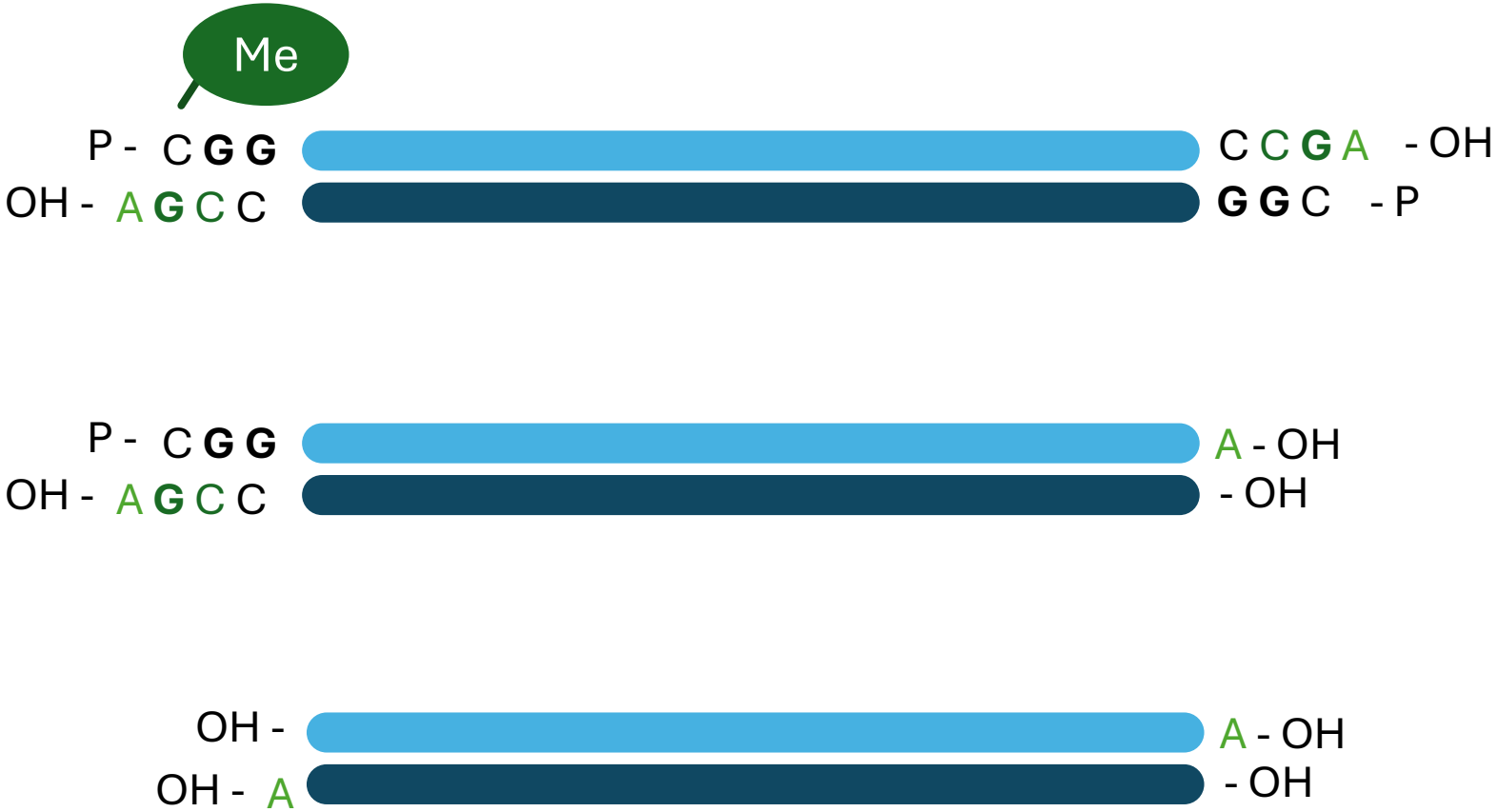
1. Dephosphorylation



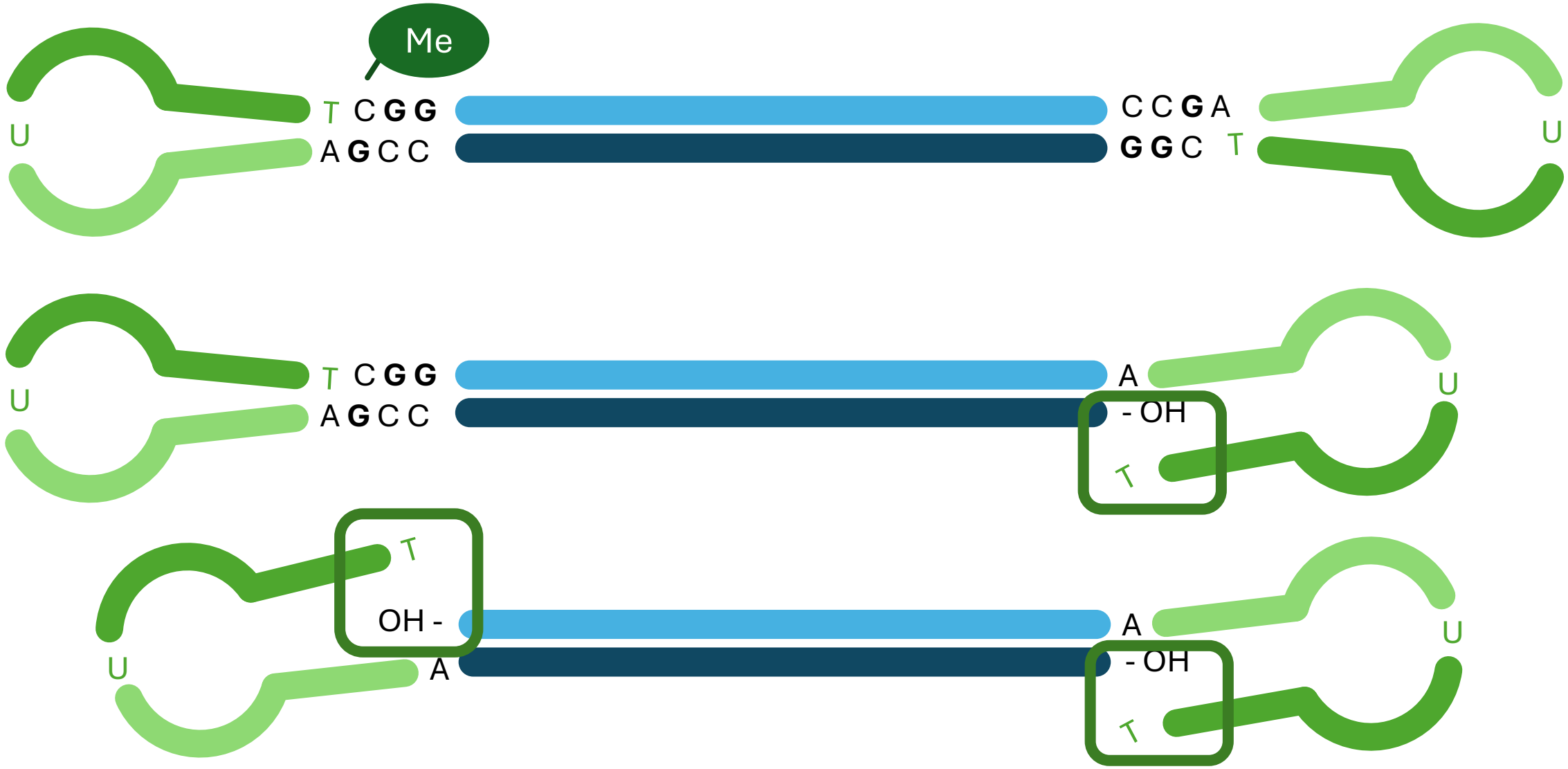
1. Dephosphorylation

2. Msp1 digestion, filling-in & dA-tailing

2. Msp1 digestion, filling-in & dA-tailing



3. Hairpin adapter ligation



4. Exonuclease degradation

4. Exonuclease degradation

5. Adapter opening

5. Adapter opening

6. Bisulfite conversion

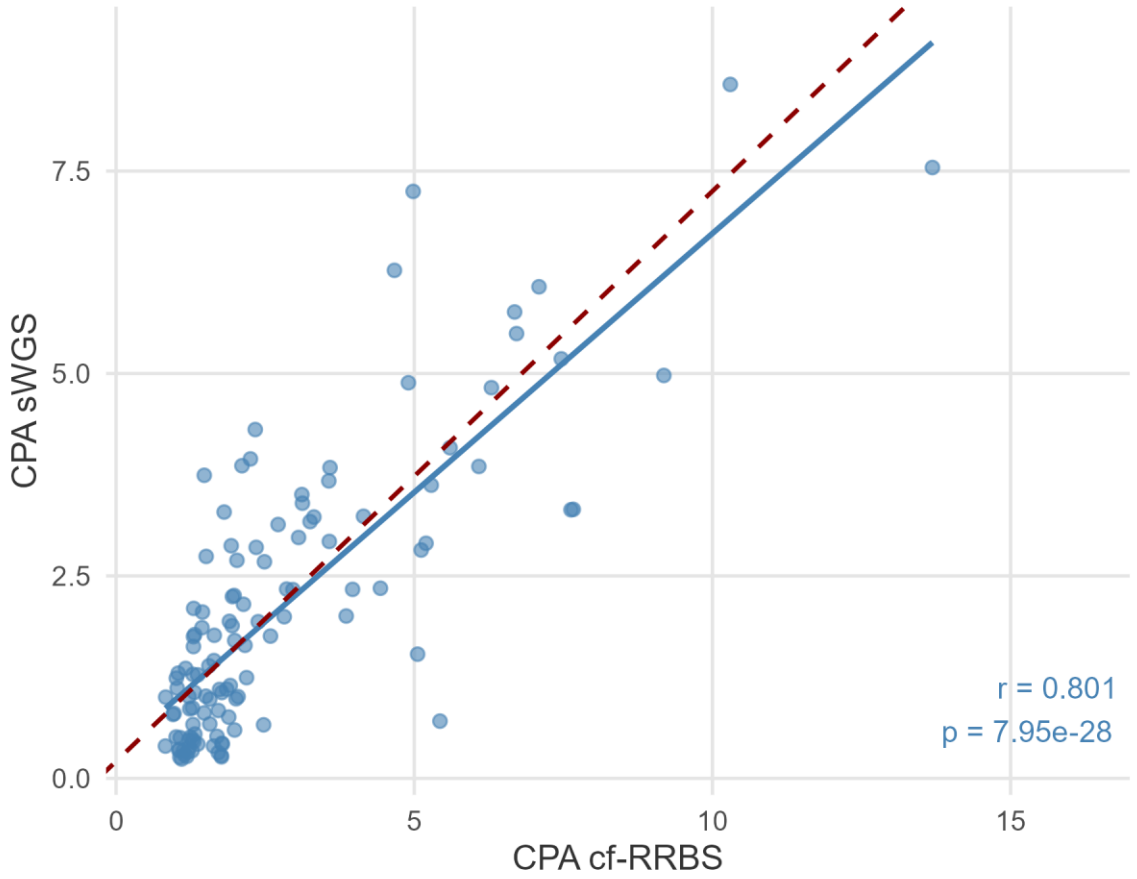
5. Adapter opening

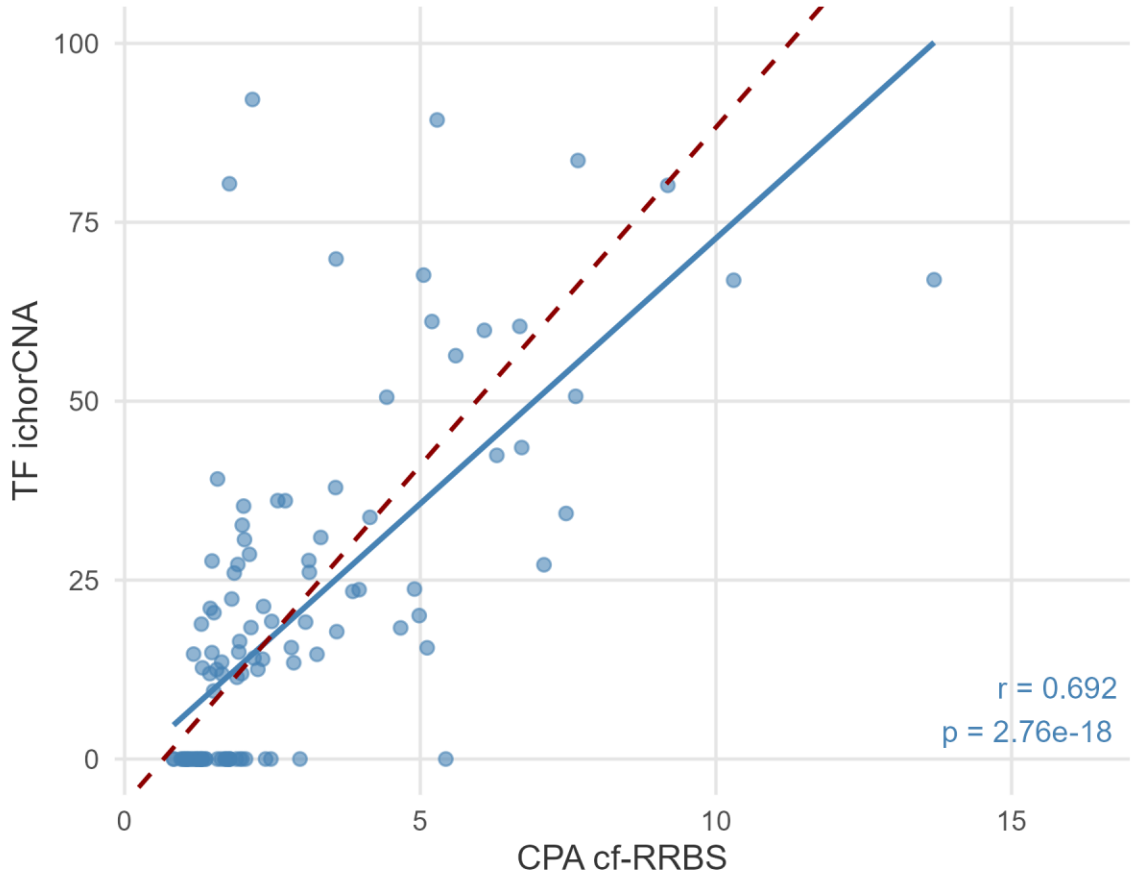
6. Bisulfite conversion

7. PCR amplification

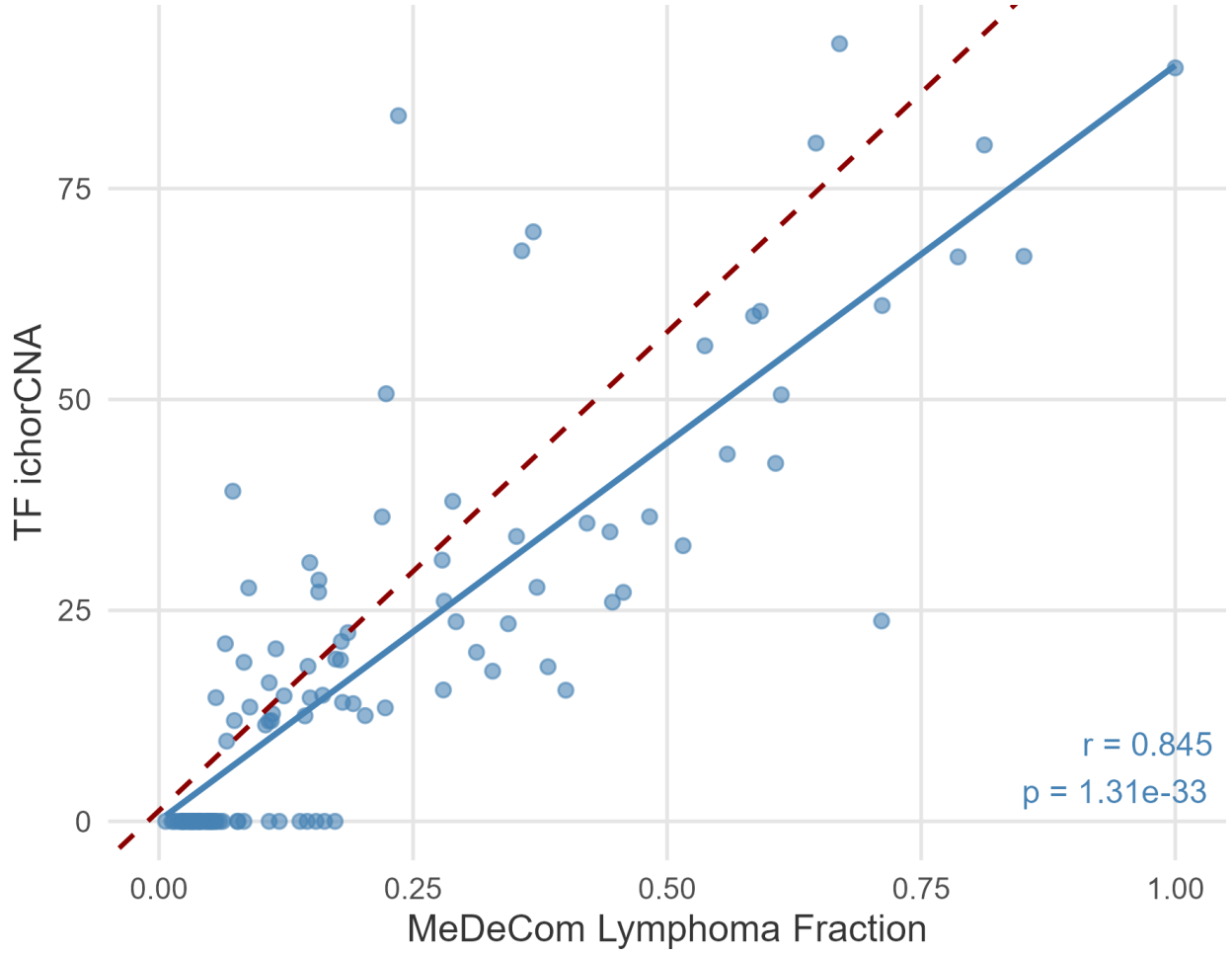
	WGBS	cf-RRBS
Coverage	~90–95% of CpGs genome-wide	~5–10% of CpGs, focused on CpG-rich regions
Input DNA requirement	Typically ≥ 100 ng	Optimized for **very low input** (~1–10 ng)
Cost per sample	~€1,500–€2,500	~€200–€500
Sequencing depth requirement	Very high (10^8 reads for adequate coverage)	Moderate (10^7 reads)
Bias	Suffers from bisulfite conversion errors and GC-bias	Selection bias: only CpG-dense regions are interrogated

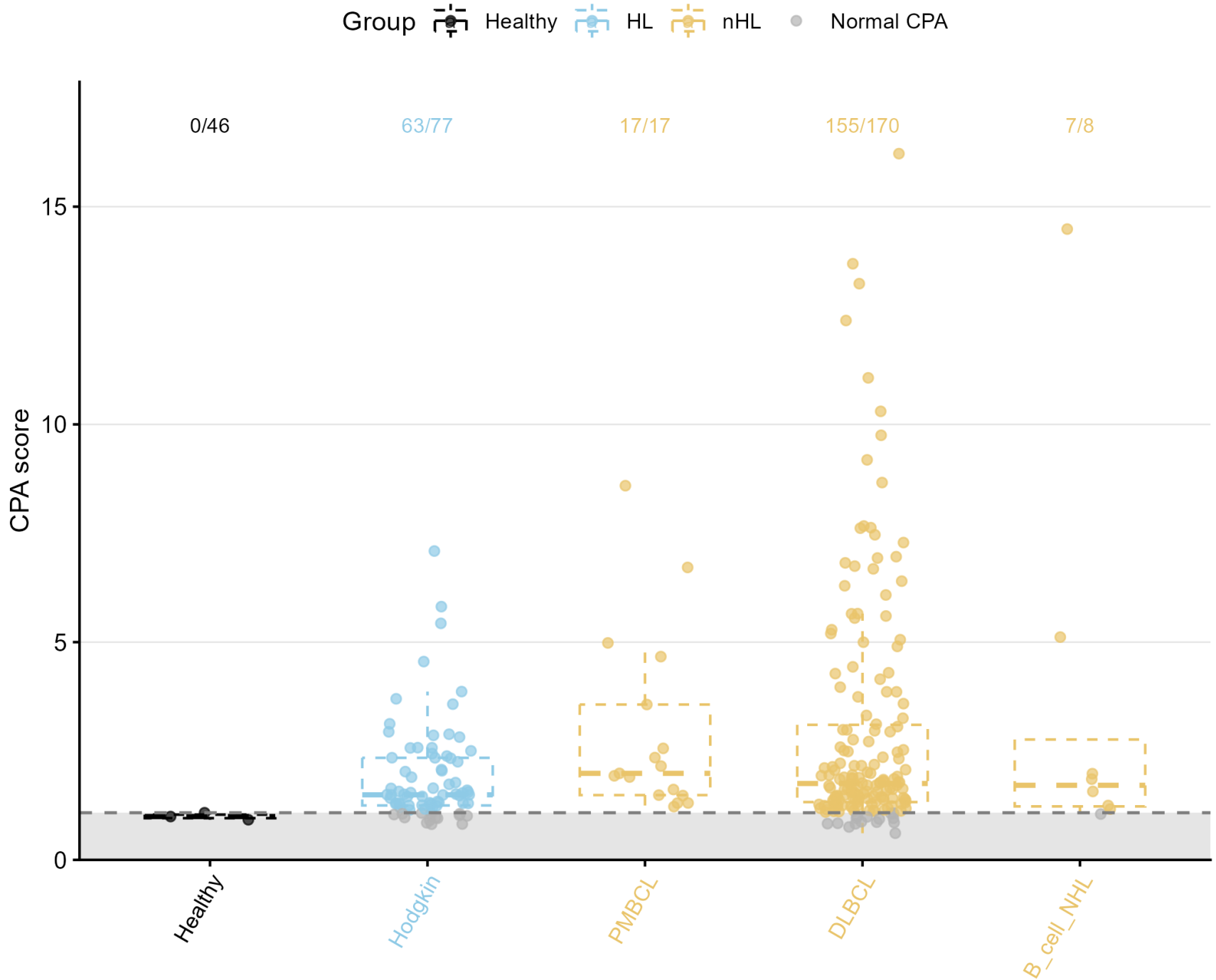
CPA scores using cf-RRBS

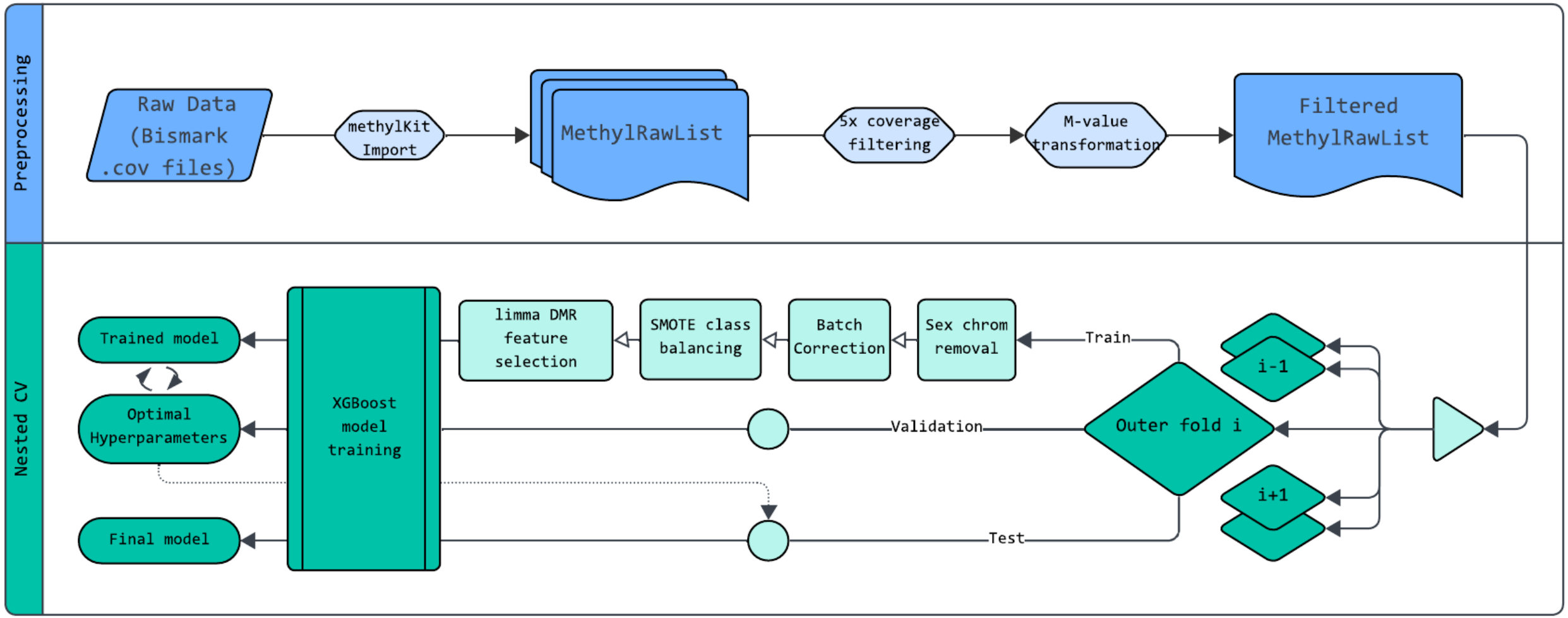


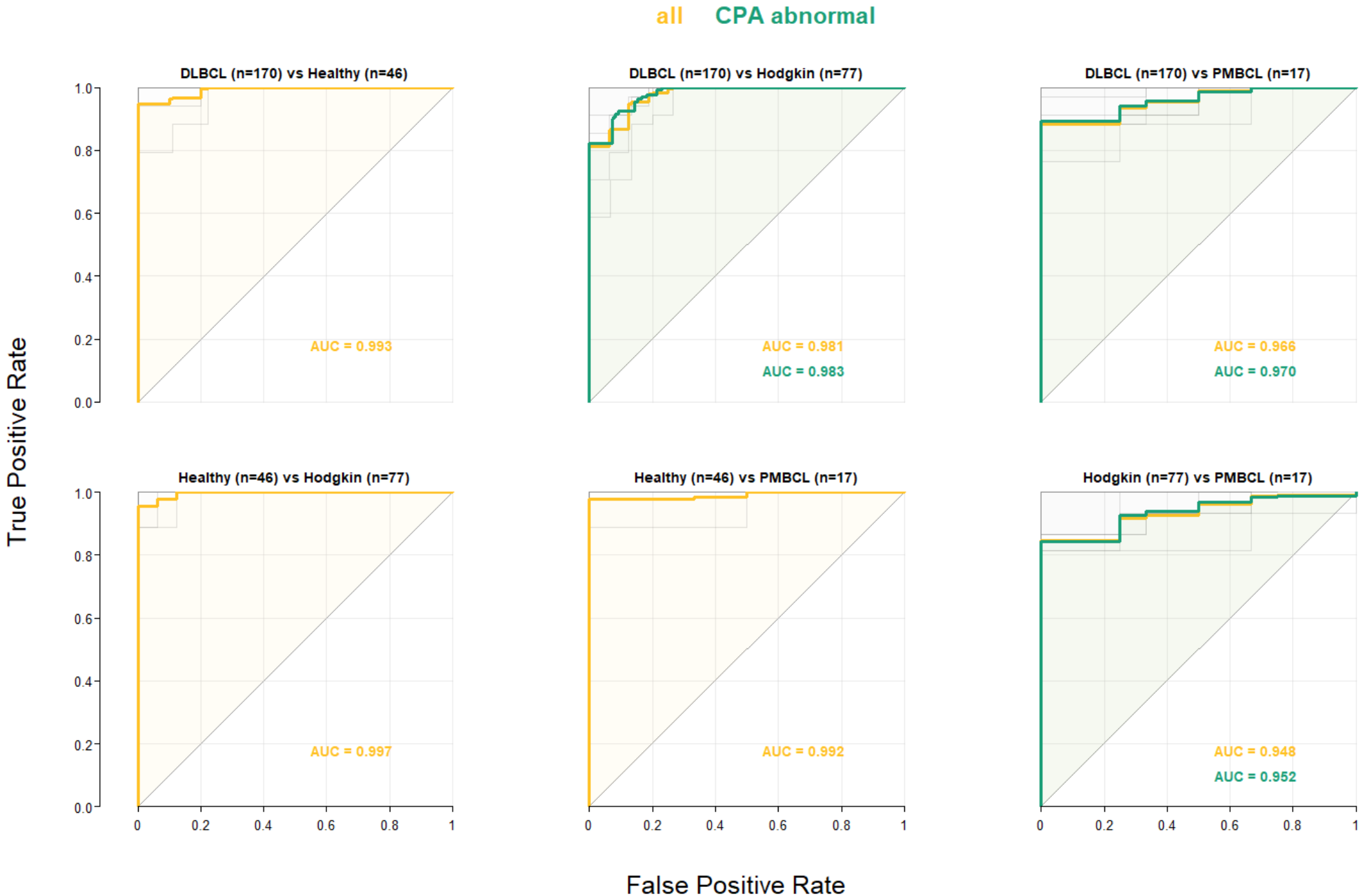


Deconvolution using cf-RRBS

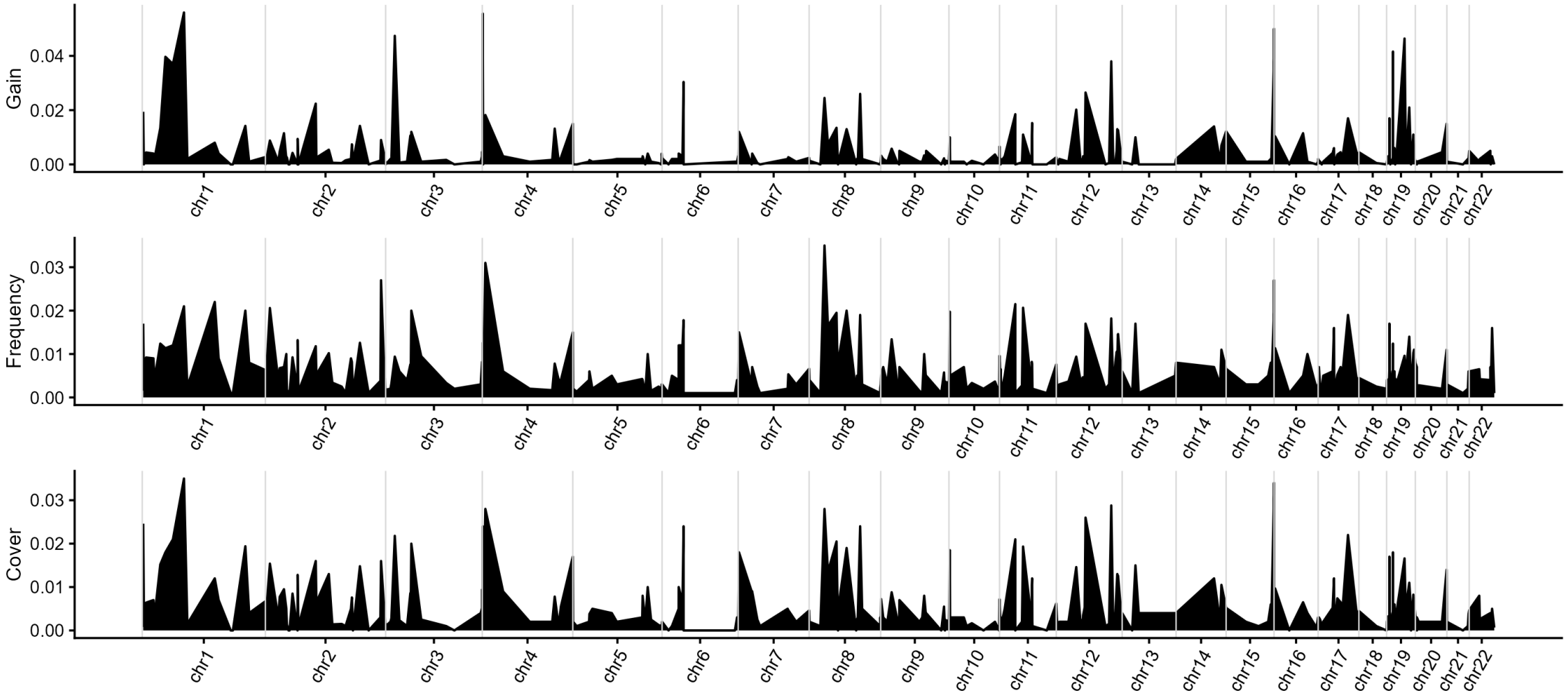



Group
Healthy
HL
nHL
Normal CPA
0/46
63/77
17/17
155/170
7/8
CPA score
15
10
5
0
Healthy
Hodgkin
PMBCL
DLBCL
B_cell_NHL


Preprocessing
Raw Data (Bismark .cov files)
methylKit Import
MethylRawList
5x coverage filtering
M-value transformation
Filtered MethylRawList
Nested CV
Trained model
Optimal Hyperparameters
Final model
XGBoost model training
limma DMR feature selection
SMOTE class balancing
Batch Correction
Sex chrom removal
Train
Validation
Test
Outer fold i
i-1
i+1


all
CPA abnormal
DLBCL (n=170) vs Healthy (n=46)
AUC = 0.993
DLBCL (n=170) vs Hodgkin (n=77)
AUC = 0.981
AUC = 0.983
DLBCL (n=170) vs PMBCL (n=17)
AUC = 0.966
AUC = 0.970
Healthy (n=46) vs Hodgkin (n=77)
AUC = 0.997
Healthy (n=46) vs PMBCL (n=17)
AUC = 0.992
Hodgkin (n=77) vs PMBCL (n=17)
AUC = 0.948
AUC = 0.952
True Positive Rate
False Positive Rate
0.0
0.2
0.4
0.6
0.8
1.0
0
0.2
0.4
0.6
0.8
1

Model Feature Importances



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