Erasmus MC
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ctDNA monitoring by methylated cfDNA sequencing of LpnPI digested fragments (cfMeD-seq)

Medical Oncology

Erasmus MC

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Conflict of interest

No disclosures

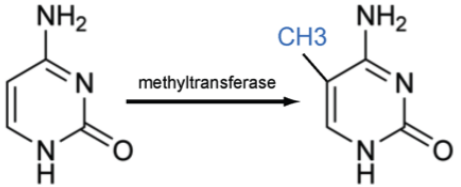
Why cfDNA methylation?

Regulation of gene expression

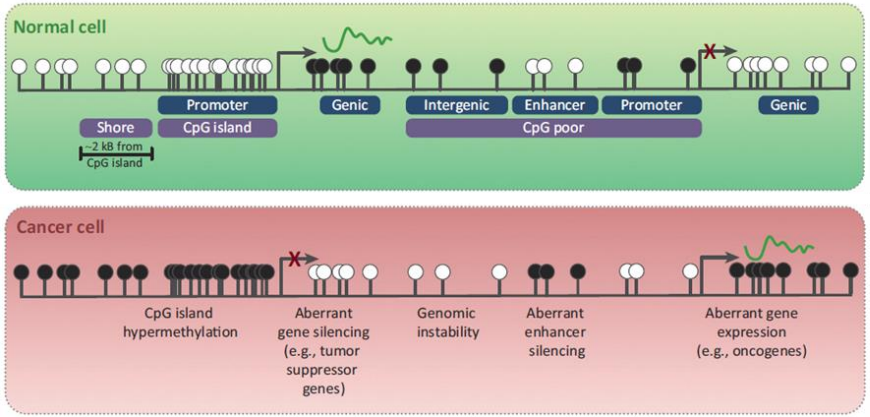
Cell type specific methylation patterns

Early changes in carcinogenesis, high penetrance

Pan-cancer & universal



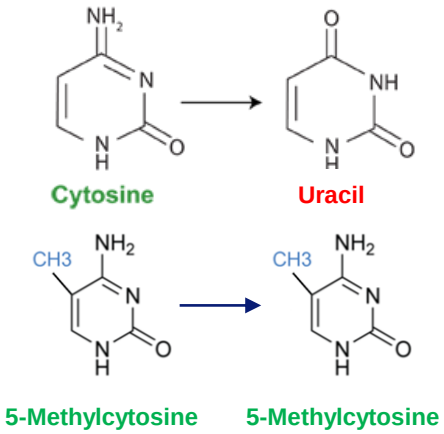
→ cfDNA methylation superior for cancer detection/classification (PMID: 33506766)



Stirzaker et al. 2014

How can we detect it?

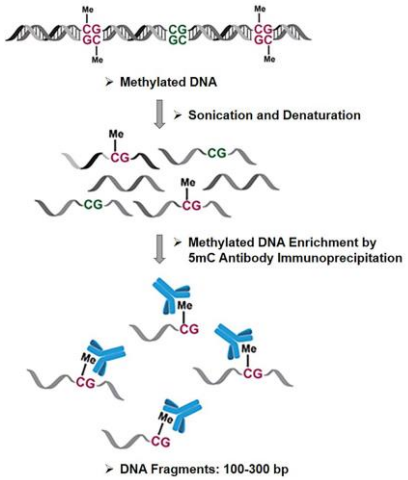
Bisulfite modification



Whole Genome
&
Reduced Representation
Bisulfite Sequencing

(WGBS & RRBS)

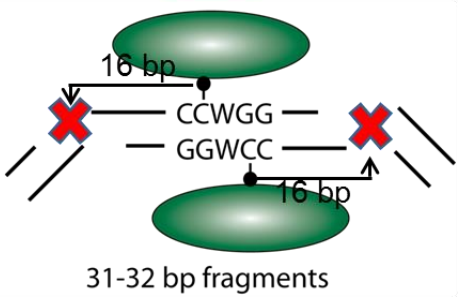
Antibodies



Methylation-dependent
immuno-precipitation or
capture

(MeDIP- & MeCap-seq)

Restriction enzymes



Methylation-Dependent
sequencing

(MeD-seq)

Pros & cons MeD-seq

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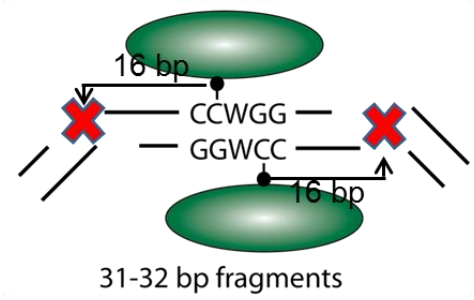
Restriction enzymes



Methylation-Dependent sequencing

(MeD-seq)

Pros:
Low input (5-10ng)
~30M reads
~50% of CpG sites covered
No bisulfite conversion (easy mapping)

Cons:
Only methylated DNA sequenced
Restriction enzyme (influenced by buffer)

MeD-seq wetlab

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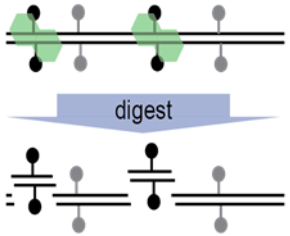
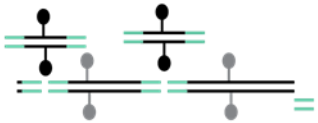
LpnPI digest

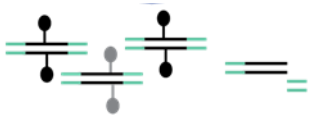


No over-digestion

Adaptors



Size selection

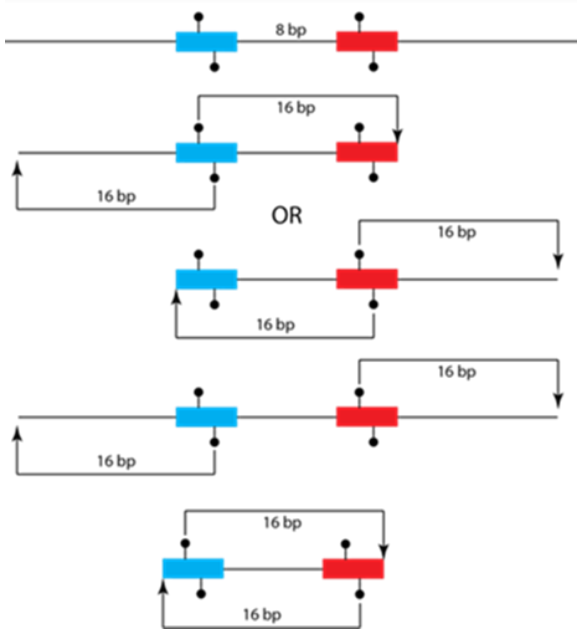


148-192 bp
32 bp + adapters

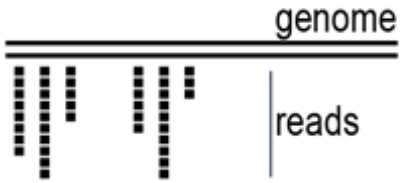
PCR & sequencing

MeD-seq bio-informatics

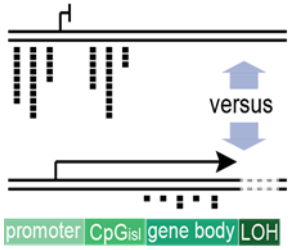
LpnPI filter



Alignment



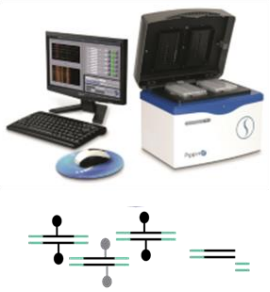
Analysis



Group per CpG Island
Group per gene (TSS)

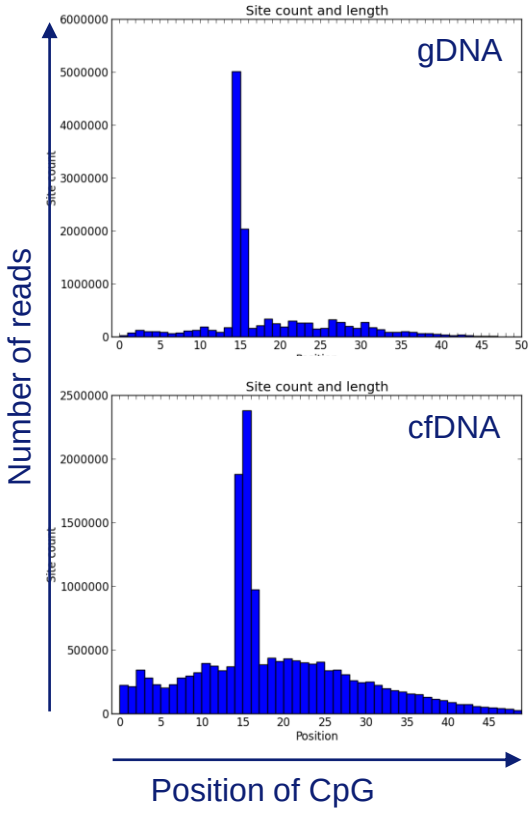
cfDNA challenges: fragmented DNA

Size selection

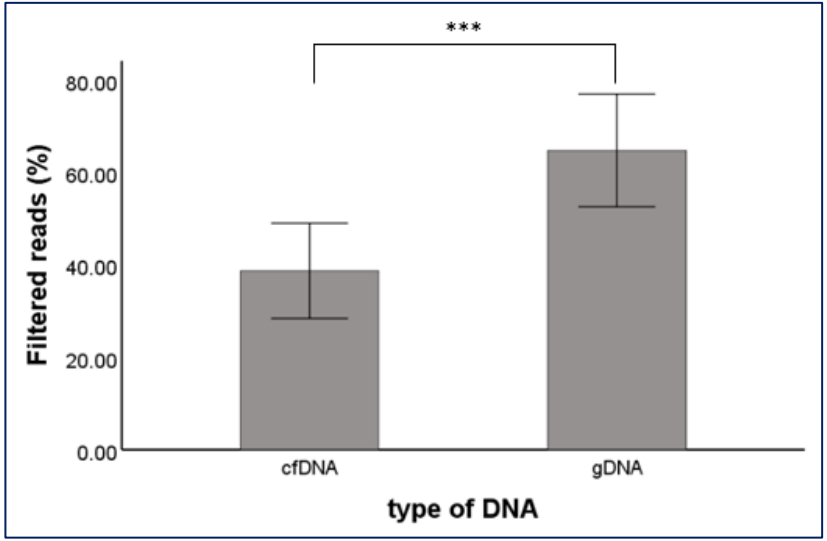


148-192 bp
32 bp + adaptors

undigested cfDNA

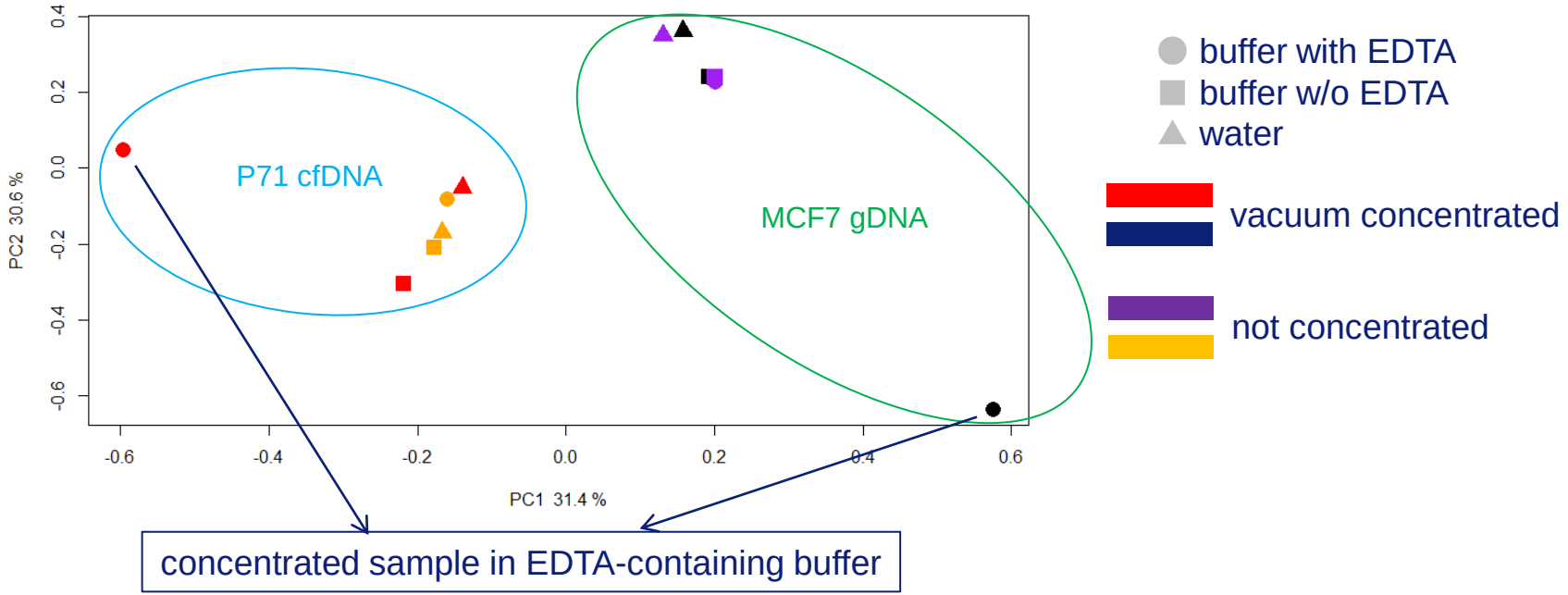


% LpnPI filtered reads



cfDNA challenges: low concentration

Current protocol requires 10 ng in 8 µl, but [cfDNA] often < 1.25 ng/µl



Vacuum concentration possible in EDTA-free buffers

cfDNA MeD-seq optimisation

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1) **Blood collection tubes**

CellSave/PAX/BCT tubes (stabilizing)	compatible
EDTA tubes	compatible

2) **cfDNA isolation method**

QiaAmp (manual, column-based)	compatible
Maxwell (semi-automated bead based)	compatible
QiaSymphony (semi-automated bead-based)	not compatible?

3) **Elution buffers**

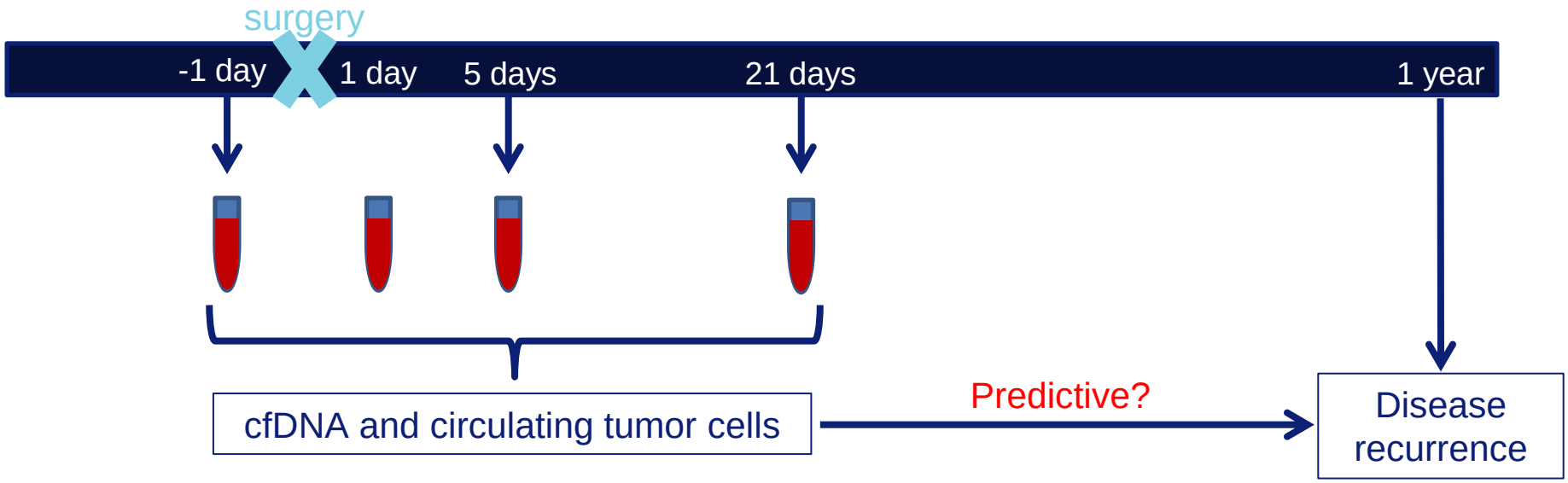
AVE (QA); ATE (QS); Maxwell; water	compatible

4) **Minimal input amount** 5-10 ng

5) **Sample concentration by speedvac** yes, without EDTA

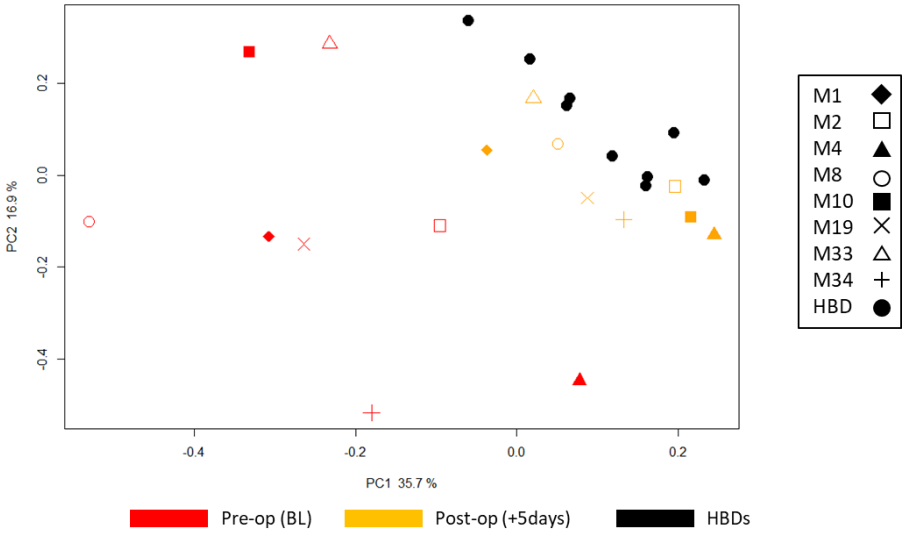
Proof of principle with clinical samples

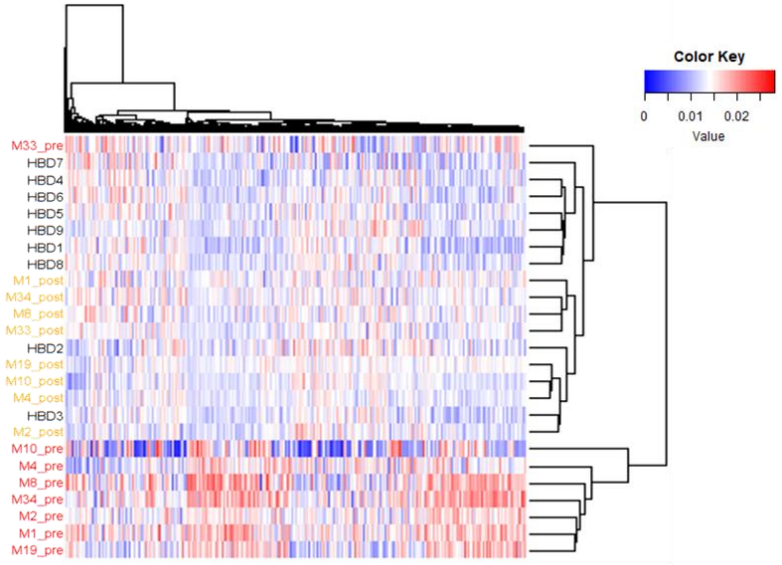
MIRACLE: 240 patients with resectable colorectal liver metastases (CRLM)



MeD-seq data before and after surgery (+5 days) for 8 patients

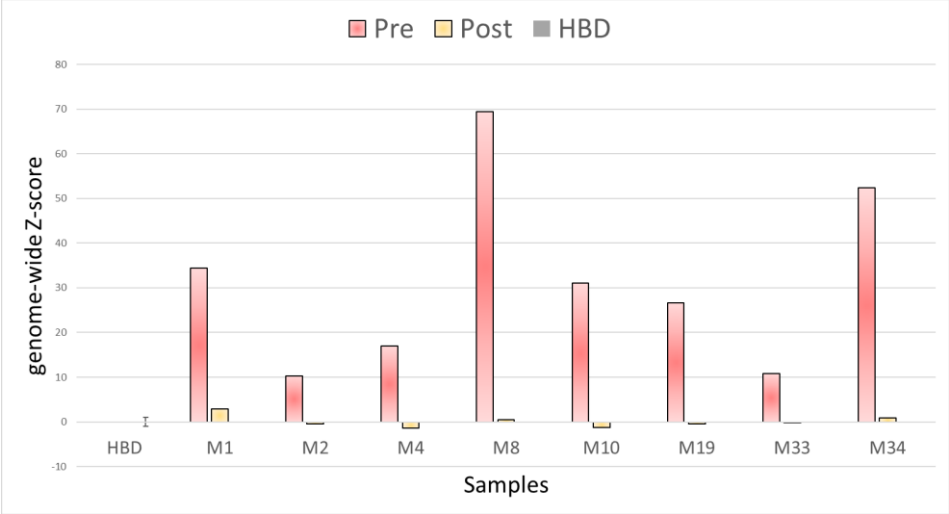
cfDNA methylation before and after surgery

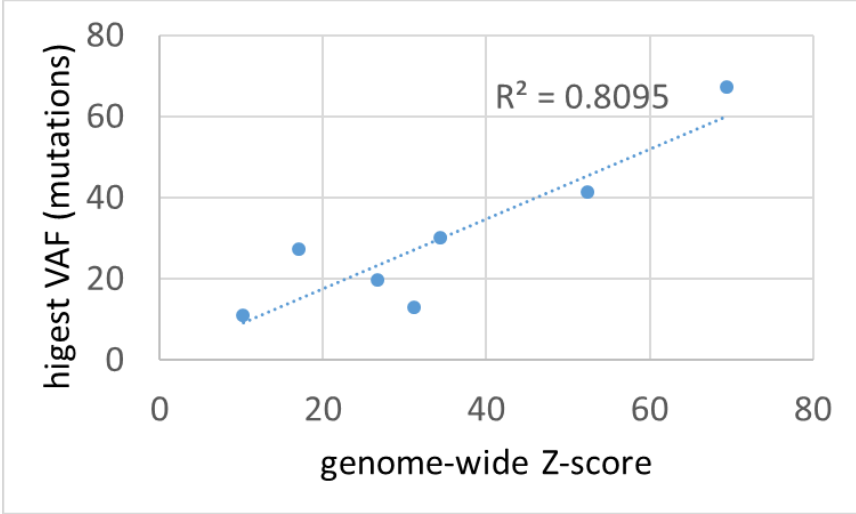




cfDNA methylation patterns in patients are different from healthy controls

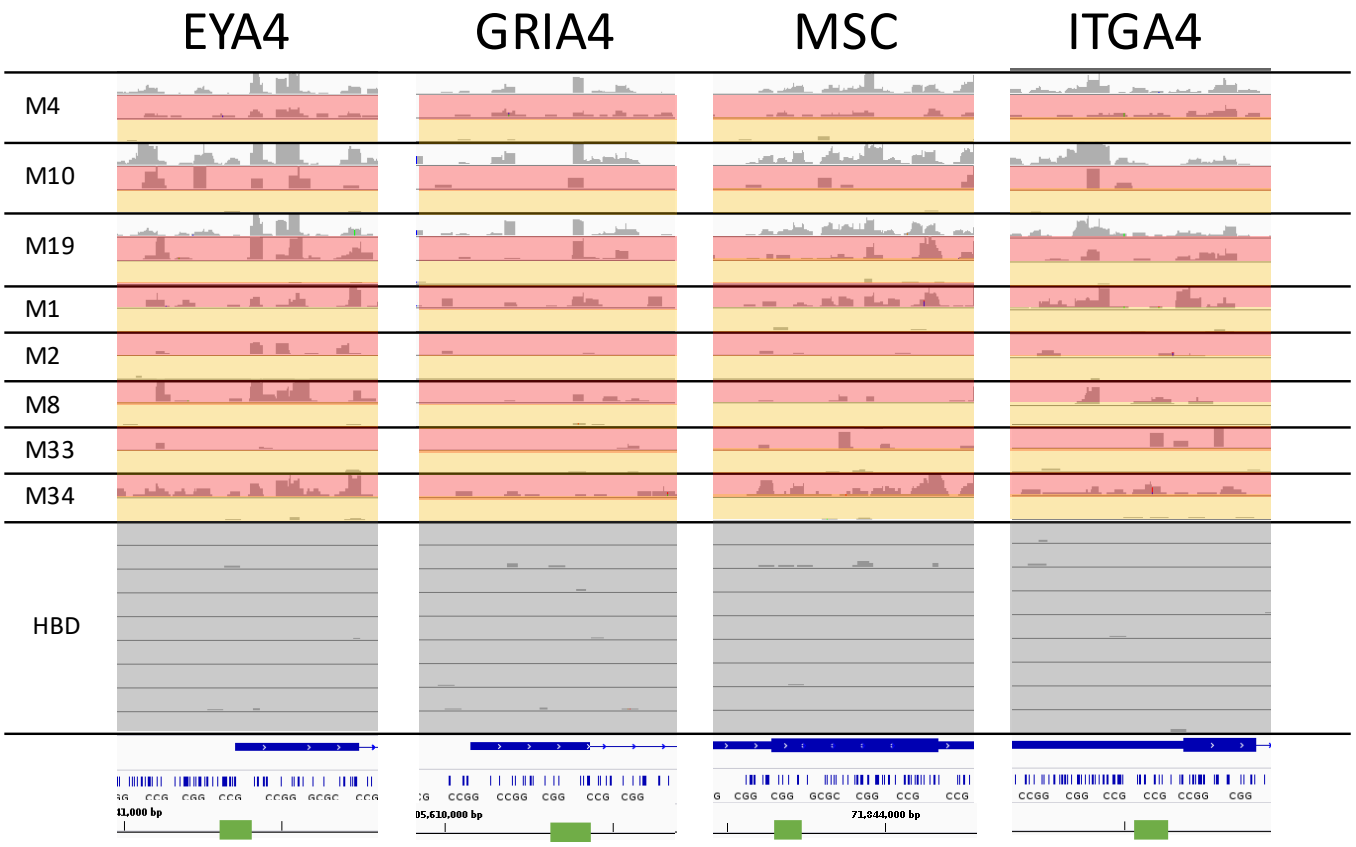
Methylation score & ctDNA load





genome-wide Z-score: total deviation in MeD-seq profile from HBDs reflects level of tumor-derived cfDNA (ctDNA) in the blood

Results for known CRC-specific markers



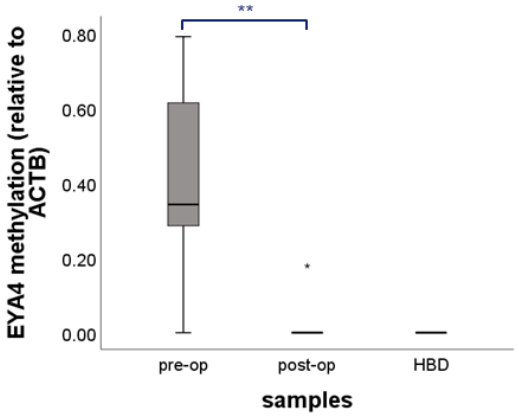
Metastatic tissue

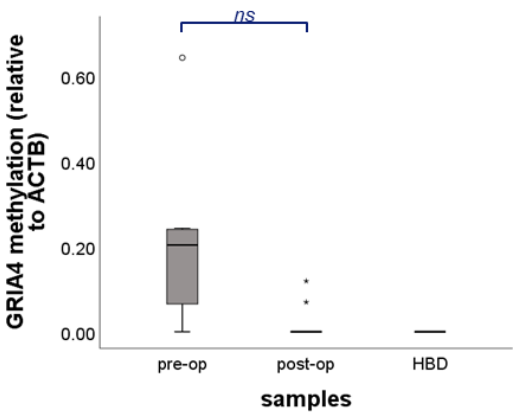
Pre-op cfDNA

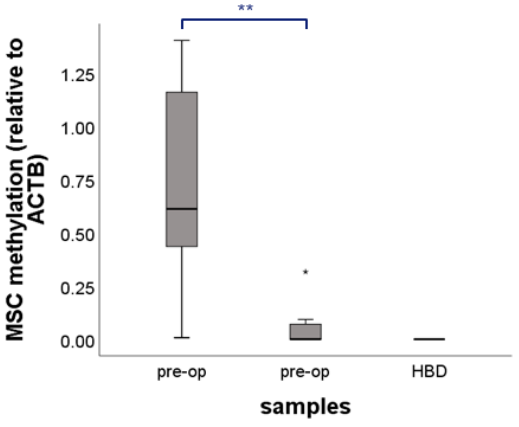
Post-op cfDNA

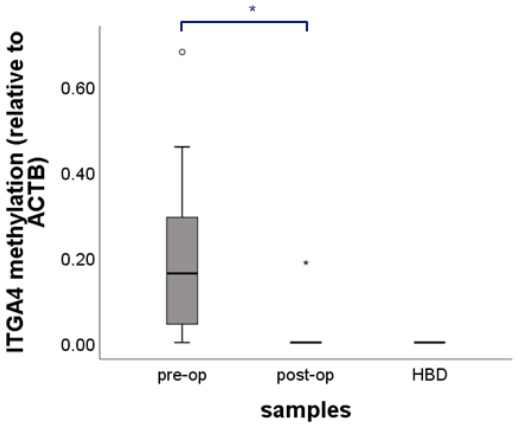
Barault et al, Gut 2018

Independent validation by qMSP





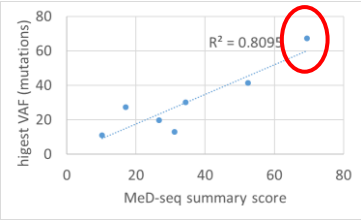


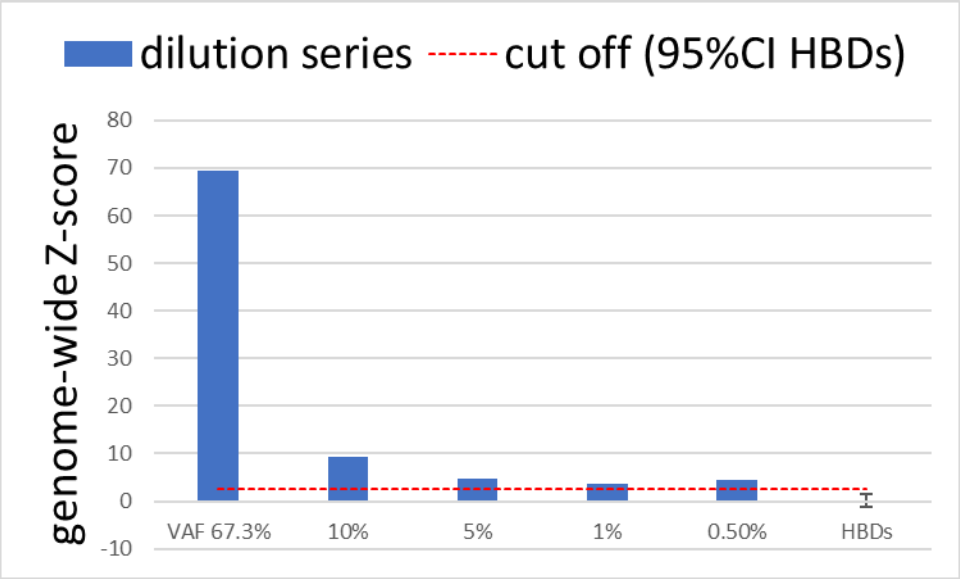


MeD-seq versus qMSP

Gene	Rho	P-value
EYA4	0.78	0.001
GRIA4	0.48	0.08
MSC	0.92	<0.001
ITGA4	0.59	0.003

How low can we go?





Genome-wide Z-score remains elevated down to 0.5%

(upper limit of 95% confidence interval of controls)

Summary & Discussion

MeD-seq represents a promising method for cfDNA methylation profiling
- works with 5-10 ng
- affordable (<500 euros per sample)
- sensitive

Results in CRLM patient samples are consistent with literature
- specific markers
- measure for ctDNA fraction (disease monitoring)

Future plans
- try to decrease the input amount
- include only LpnPI digested fragments for library preparation
- provide clinical proof of concept in MIRACLE cohort

Acknowledgements

Erasmus MC

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Vanja de Weerd
Marjolijn van der Put
Lindsay Angus
Lisanne van Dessel
Martijn Lolkema
Stefan Sleijfer
John Martens

All participating patients