DNA methylation and gene expression as determinants of genome-wide cell-free DNA fragmentation

Dr. Michaël Noë (currently at Netherlands Cancer Institute, Amsterdam)

April 4, 2025

Disclosures

Nothing to disclose

cfDNA fragmentation profiles

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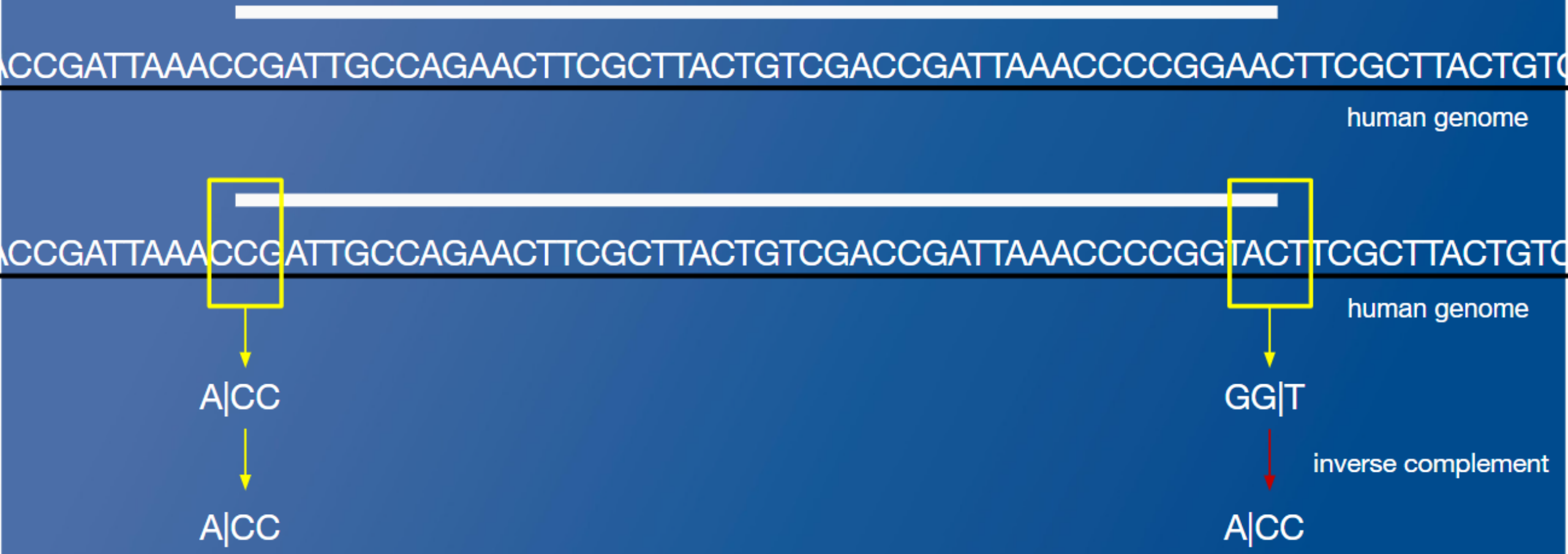
Random cfDNA fragments start- and end-positons

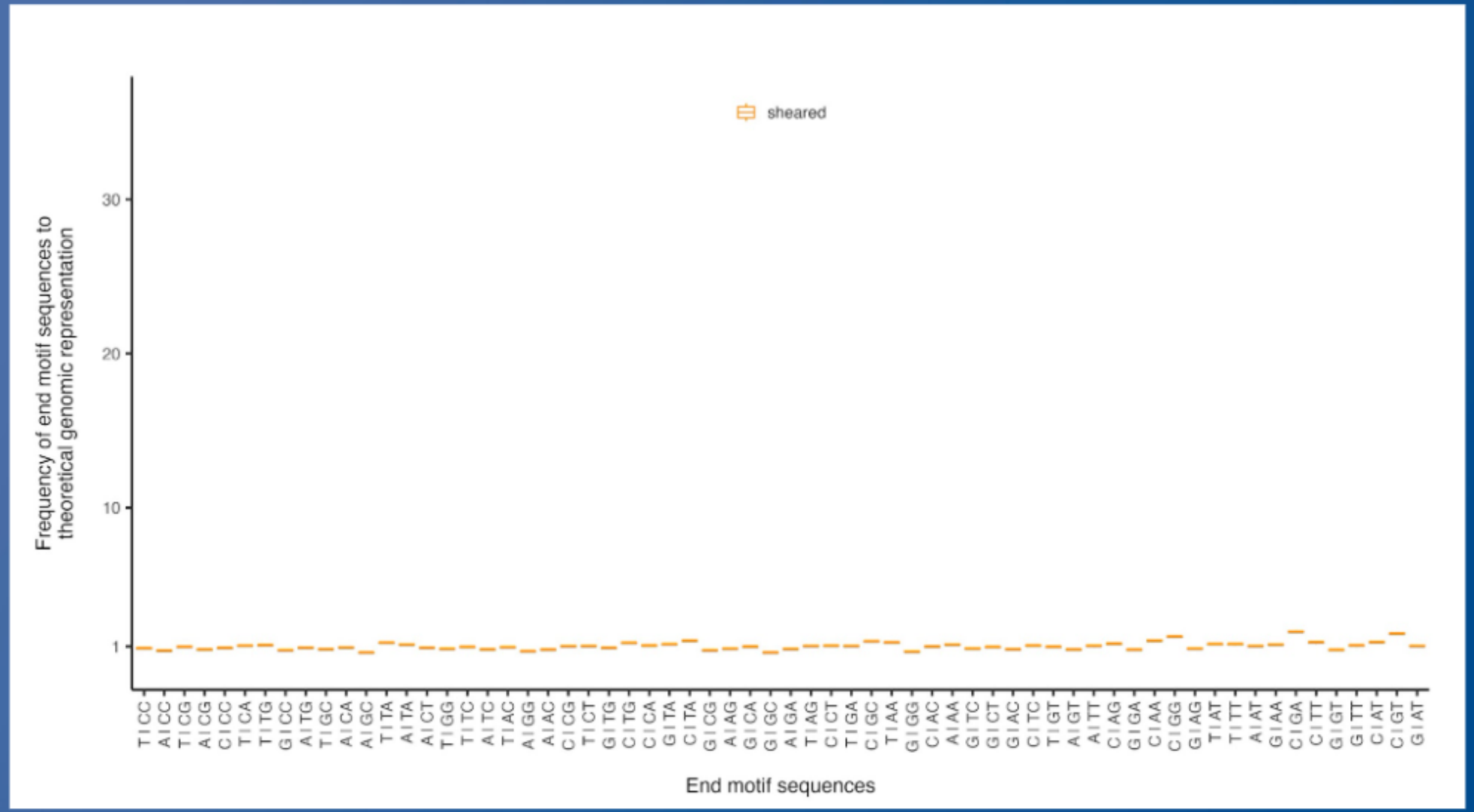
Non-random cfDNA fragment start- and end-positons

cfDNA fragmentation profiles

End-motifs



Enrichment of DNA motifs



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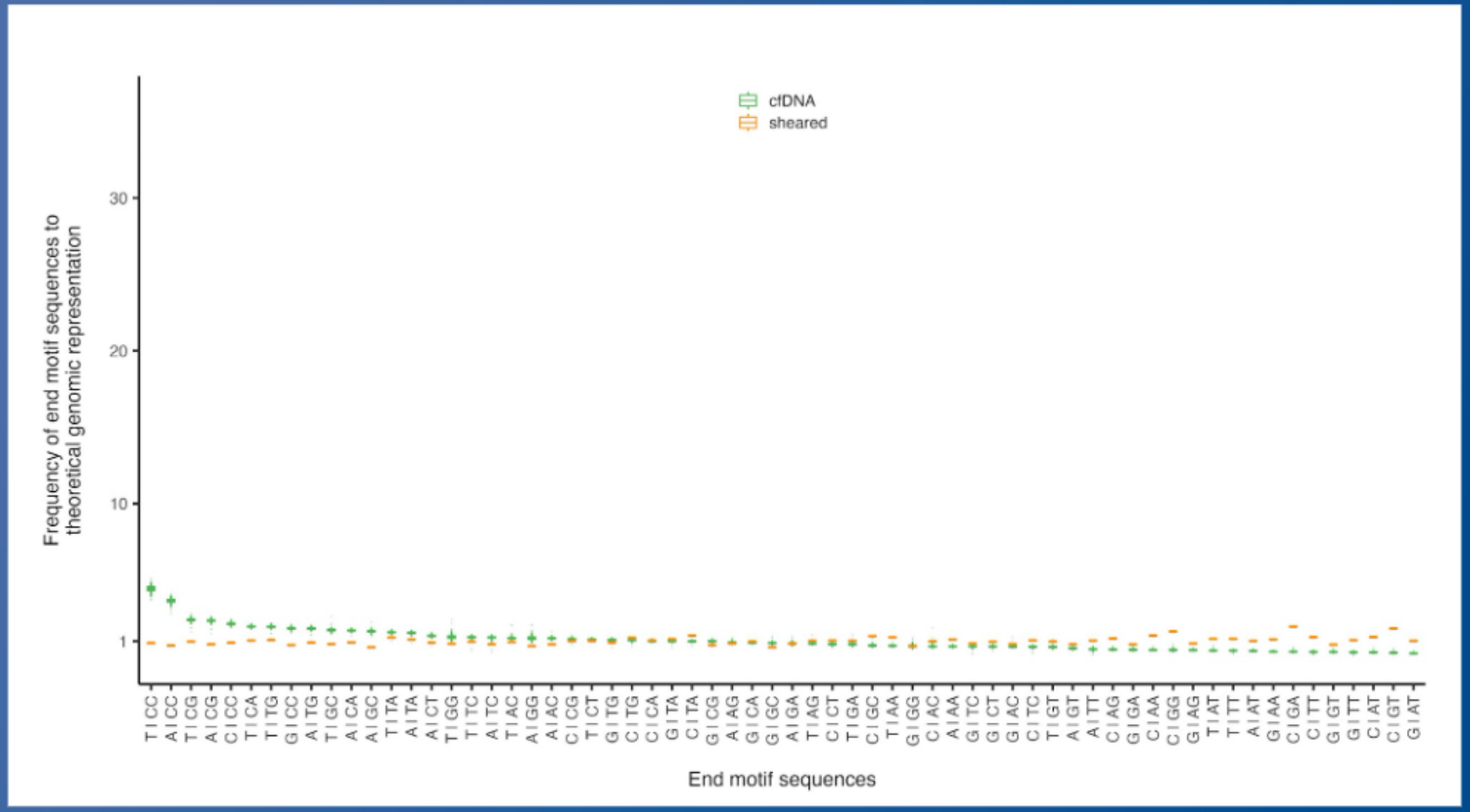

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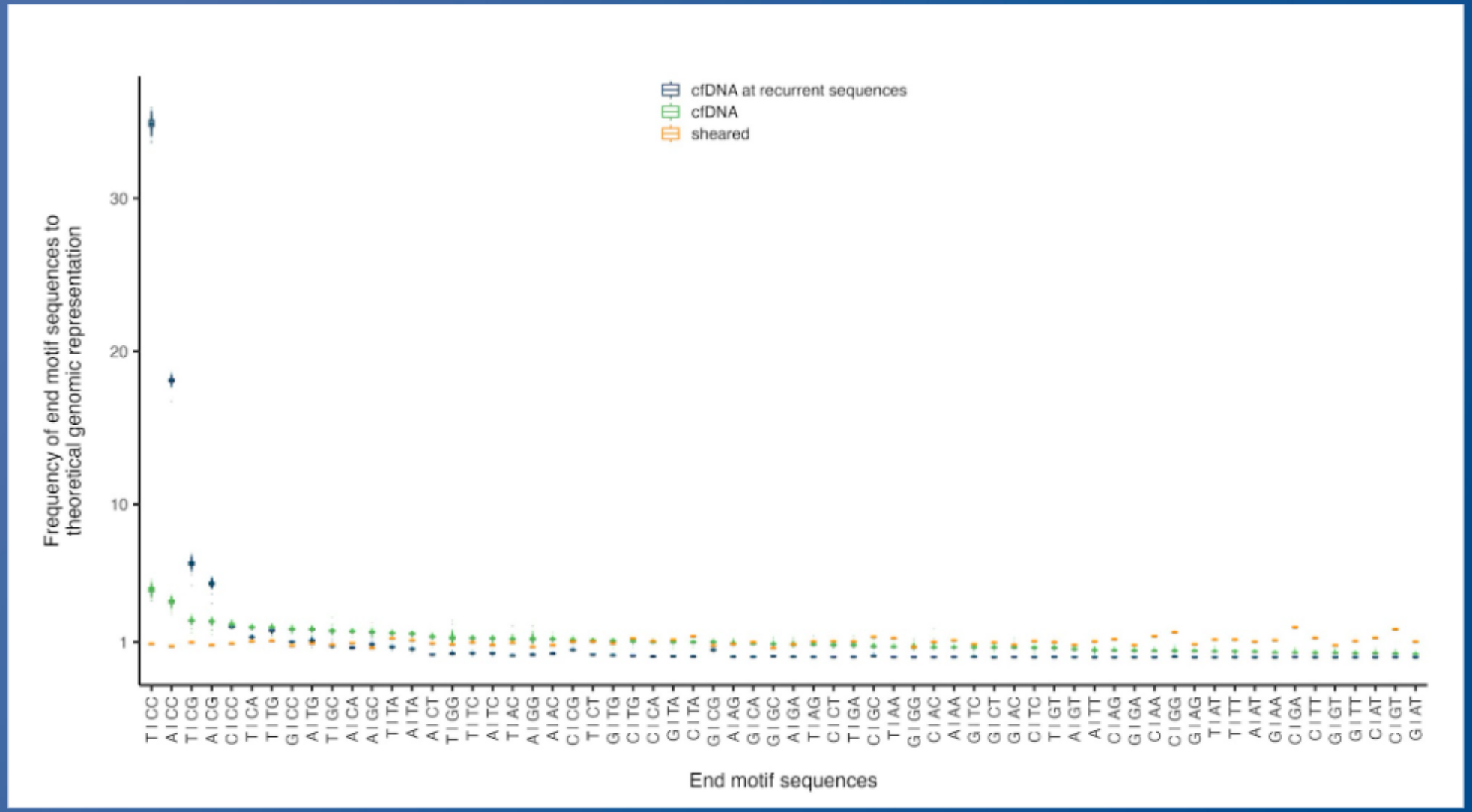

Preferred cfDNA start-sites motifs

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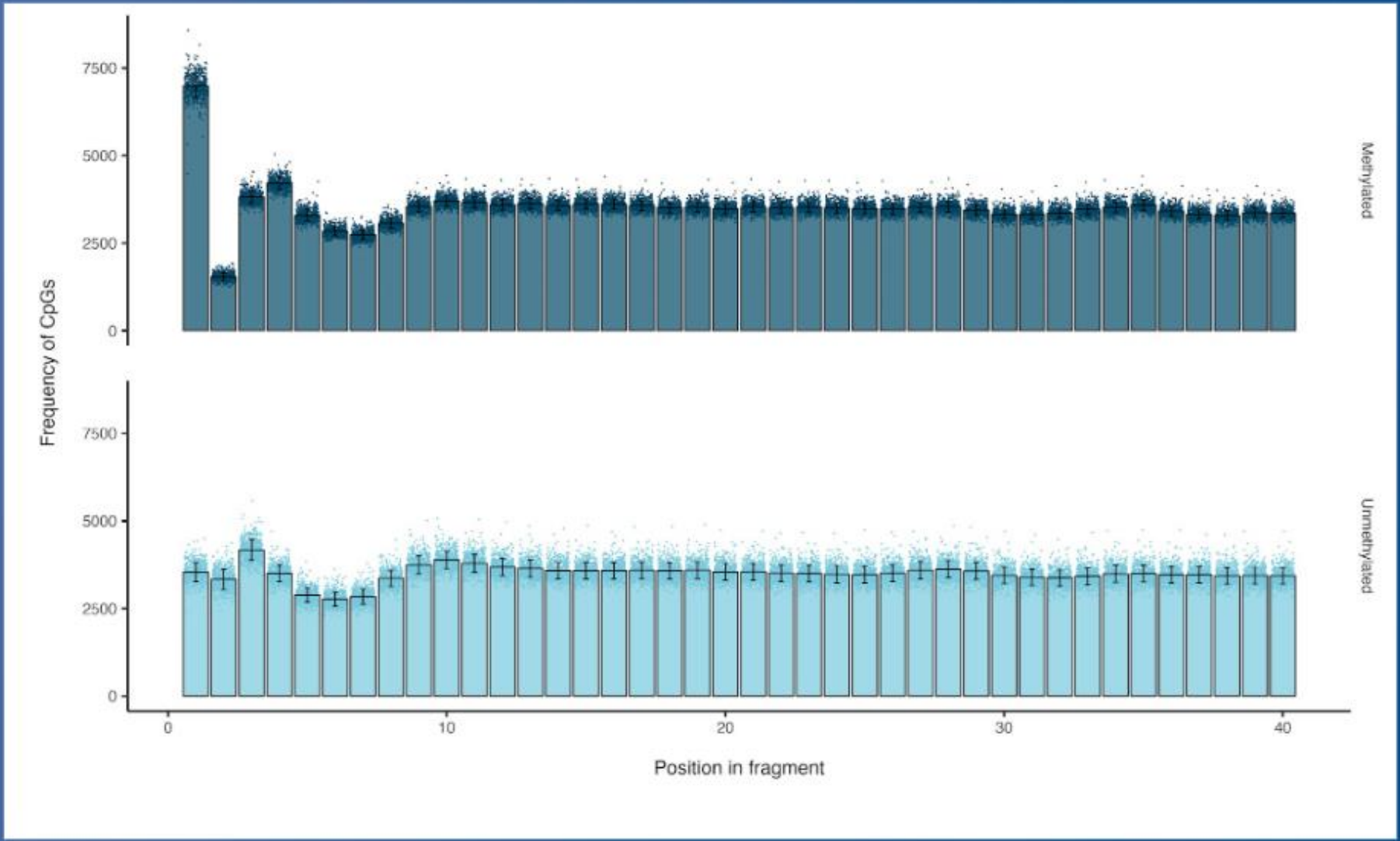
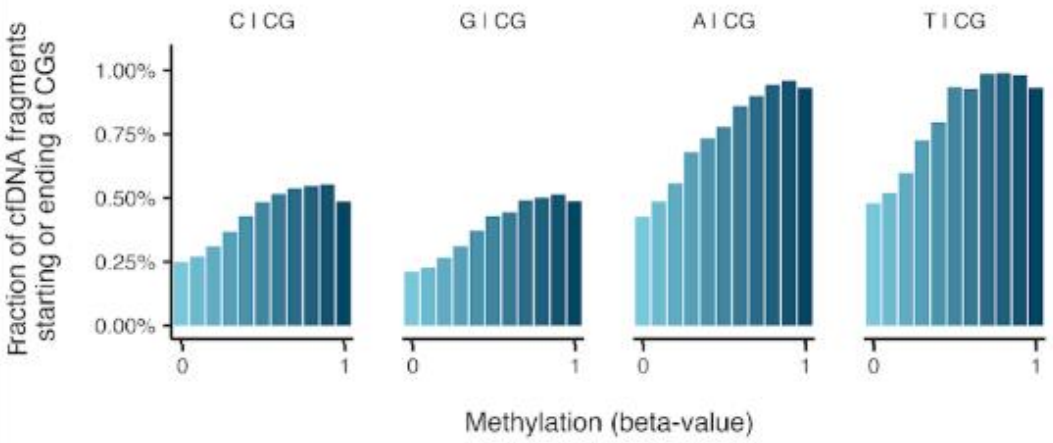
- T | CC
- A | CC
- T | CG
- A | CG

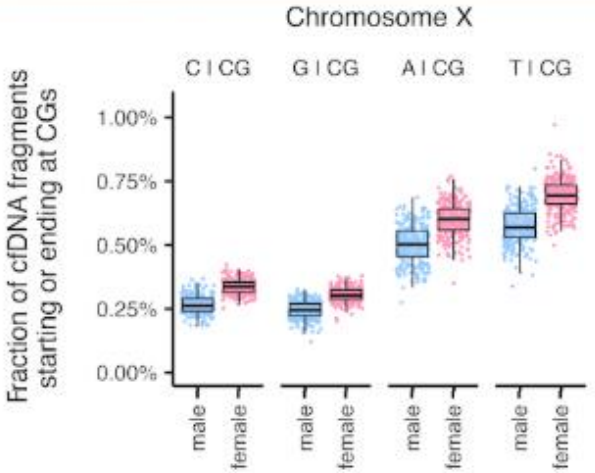
CG is target of methylation / methyl-transferases

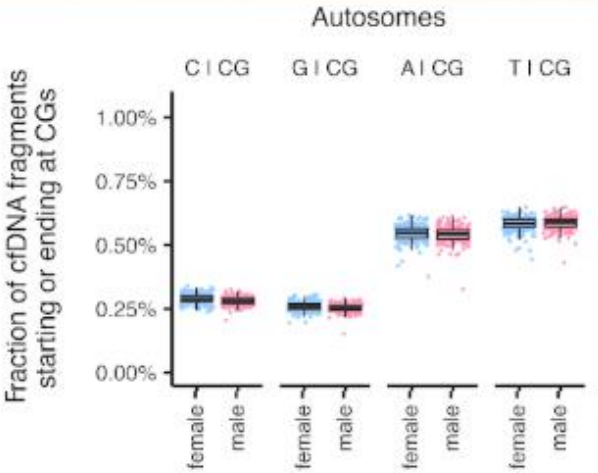
Impact of methylation on CpG-location



Impact of methylation on recurrent start-sites







Impact of methylation on recurrent start-sites

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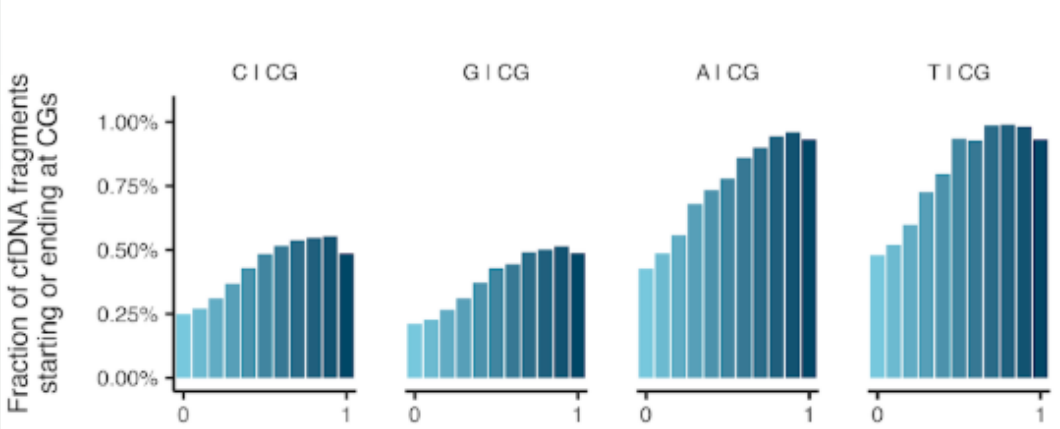
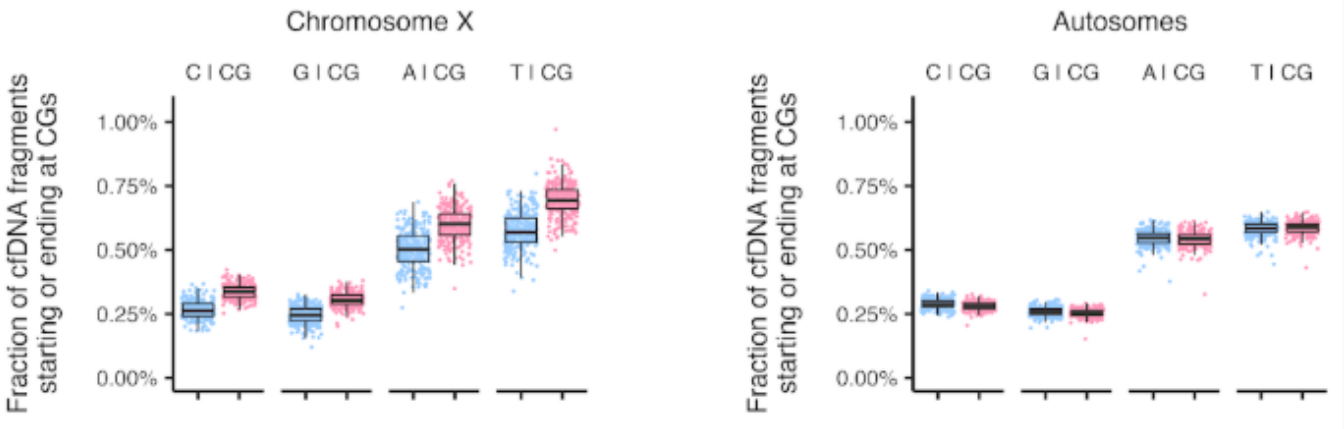
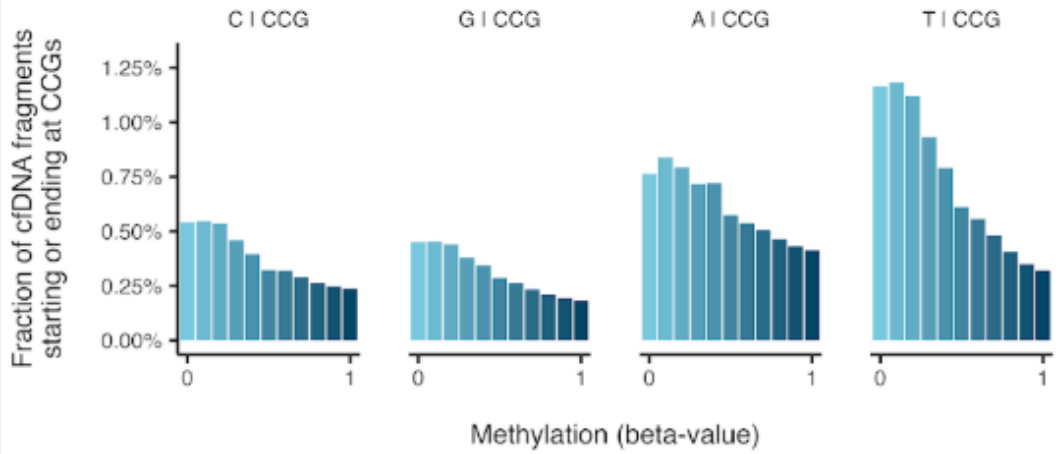
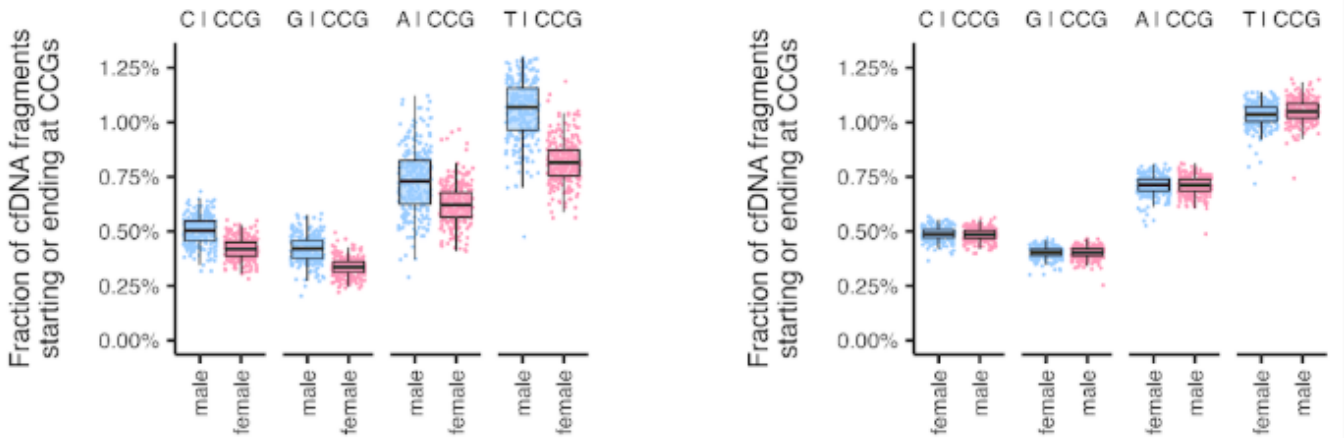




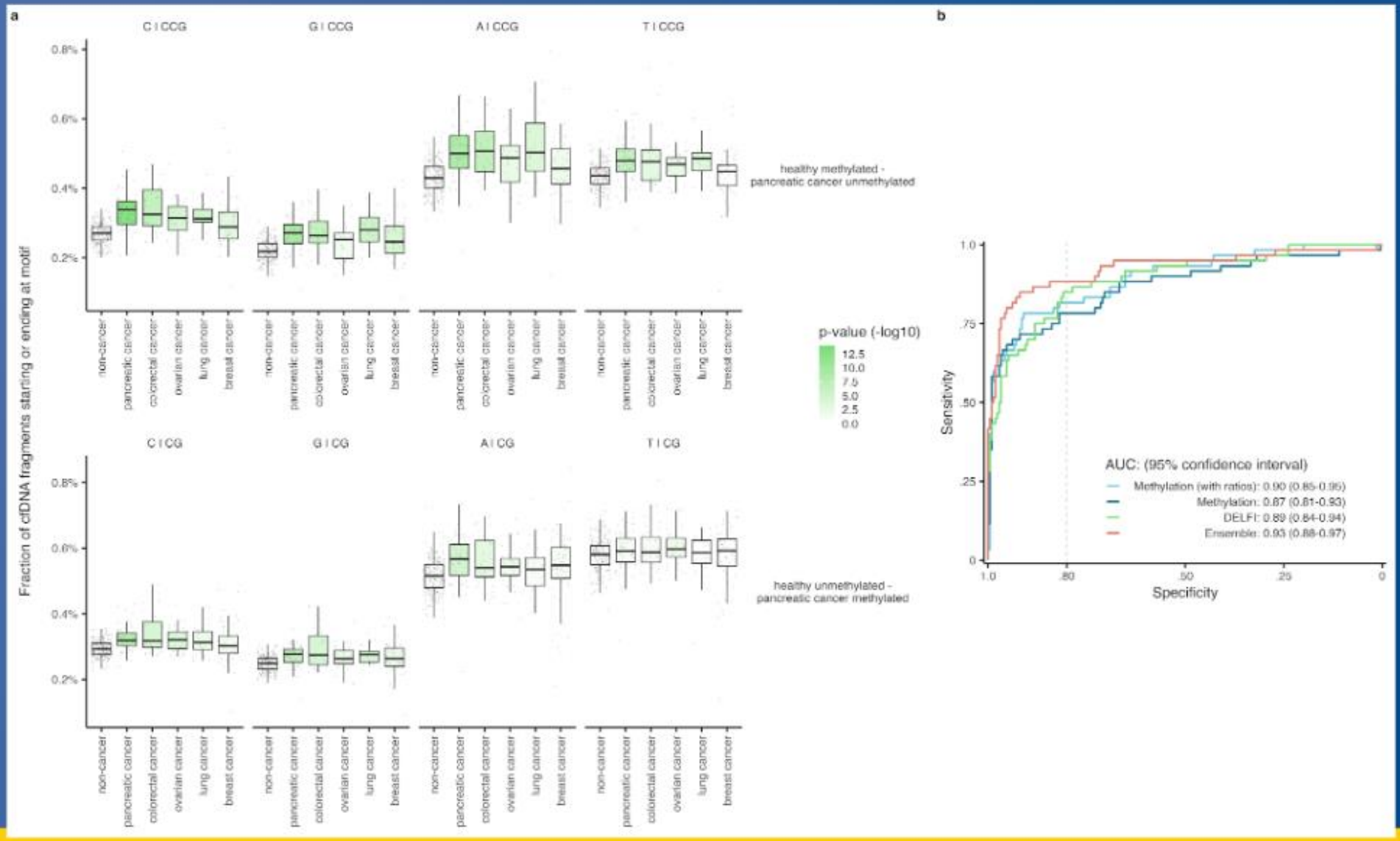




How to exploit this signal

- Finding all the differently methylated CpG's between cancer and normal cfDNA (healthy)
- Splitting in the direction of differential methylation
  - Normal cfDNA methylated / cancer unmethylated
  - Normal cfDNA unmethylated / cancer methylated
- Splitting by surrounding motif of CpG's
  - C|CG, G|CG, A|CG, T|CG
  - C|CCG, G|CCG, A|CCG, T|CCG

Detection of pancreatic cancer-specific signals



Methylation-specific cfDNA fragmentation

- Simple library-prep
- More specific for cancer-type
  - Potentially less impact from clonal haematopoiesis
  - Good alternative for tumor-informed approaches?
- Sensitive
  - Detecting cancer signals in regions where no cfDNA fragments start
- Can be used in combination with Delfi / C2i
  - Ensemble model is even more sensitive

Why does this perform well

- Increases in signal are easier to detect
  - Delfi (combination of amplifications + shorter ctDNA fragments)
  - Methylation-based fragmentation: looking in locations where no cfDNA fragments start

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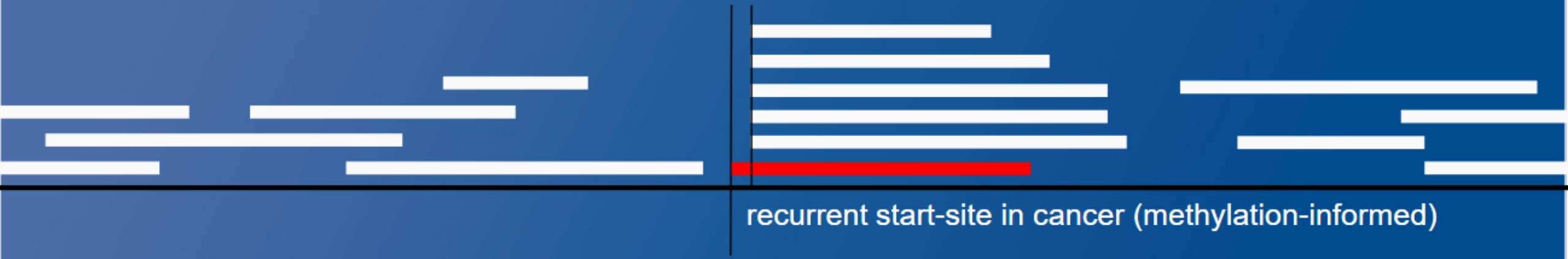
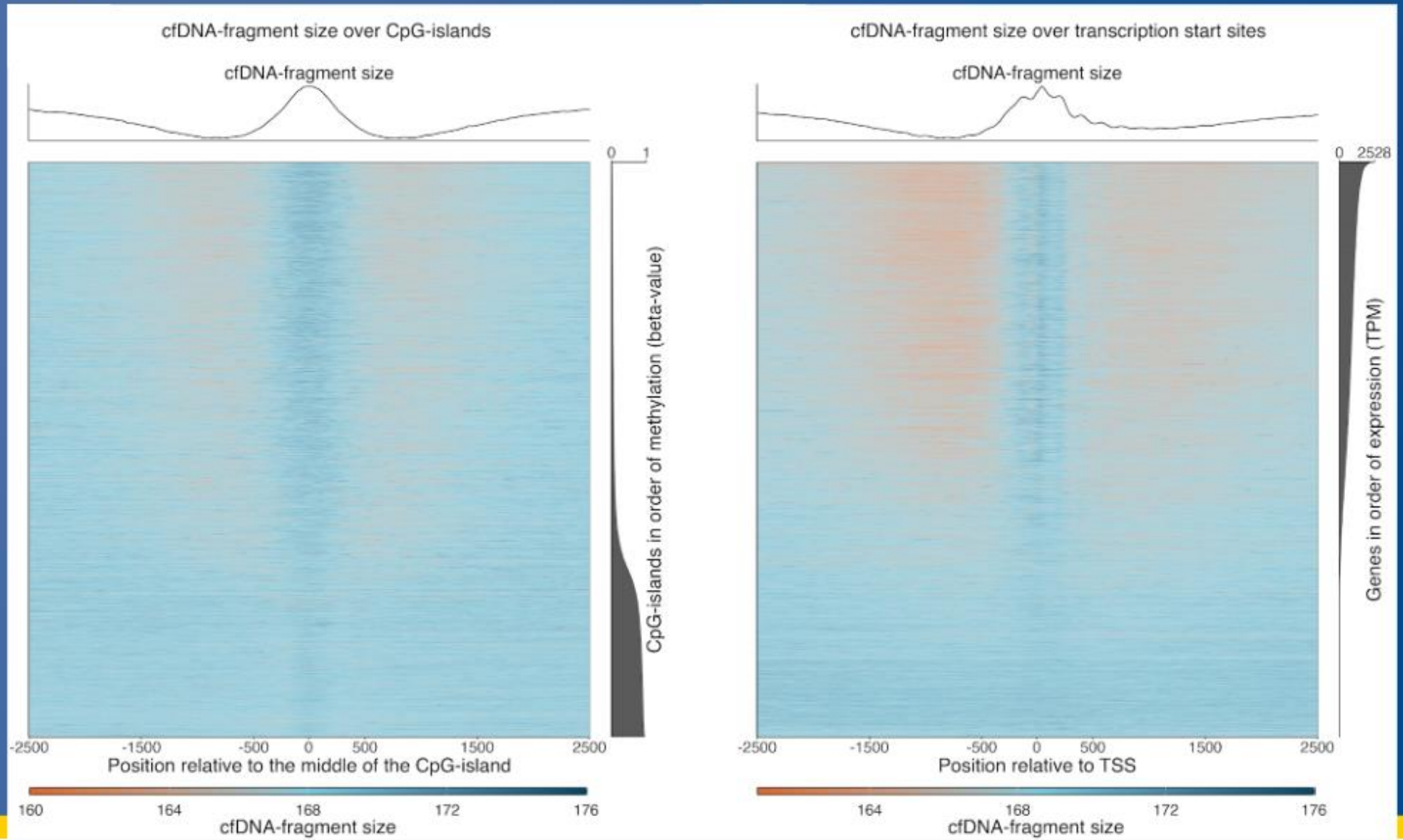


Why are tumor cfDNA fragments shorter?



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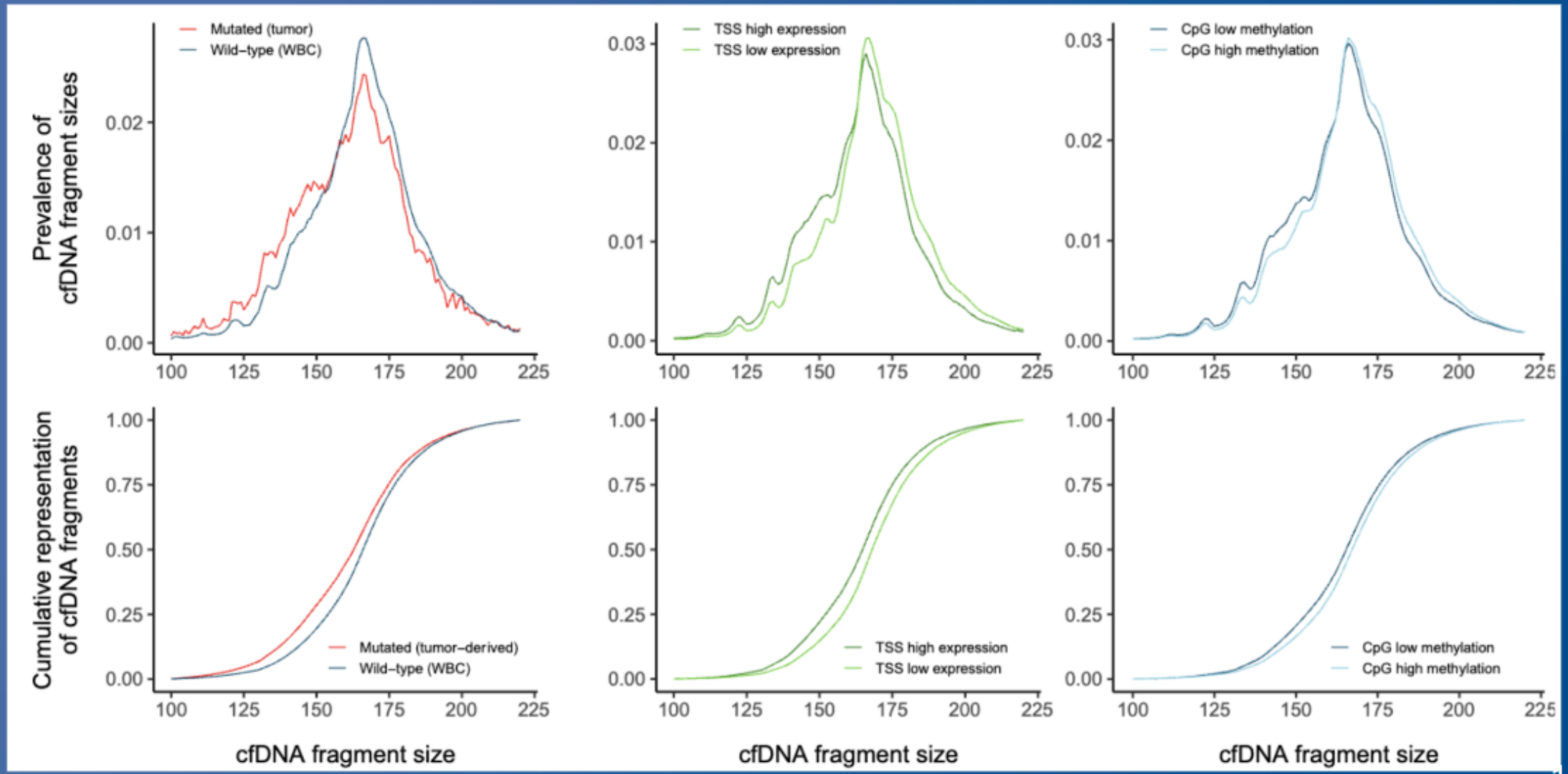

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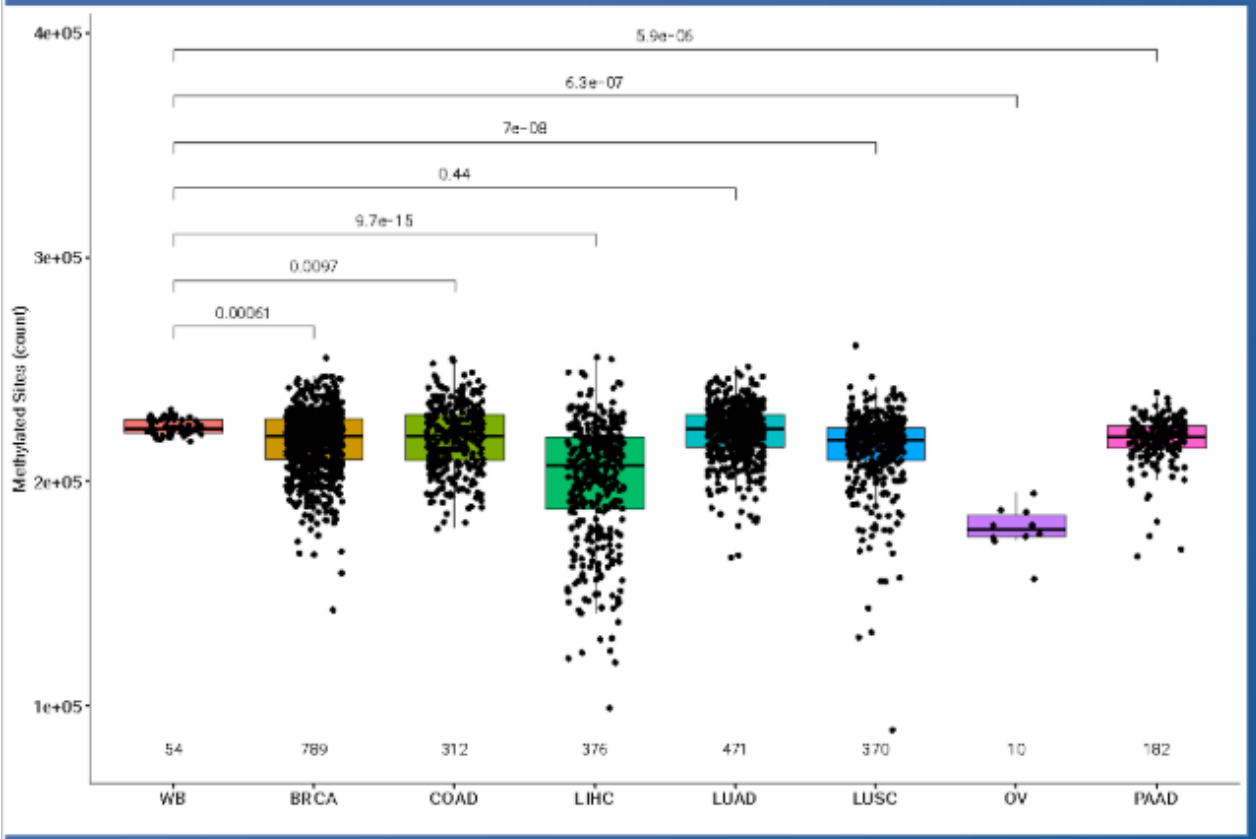
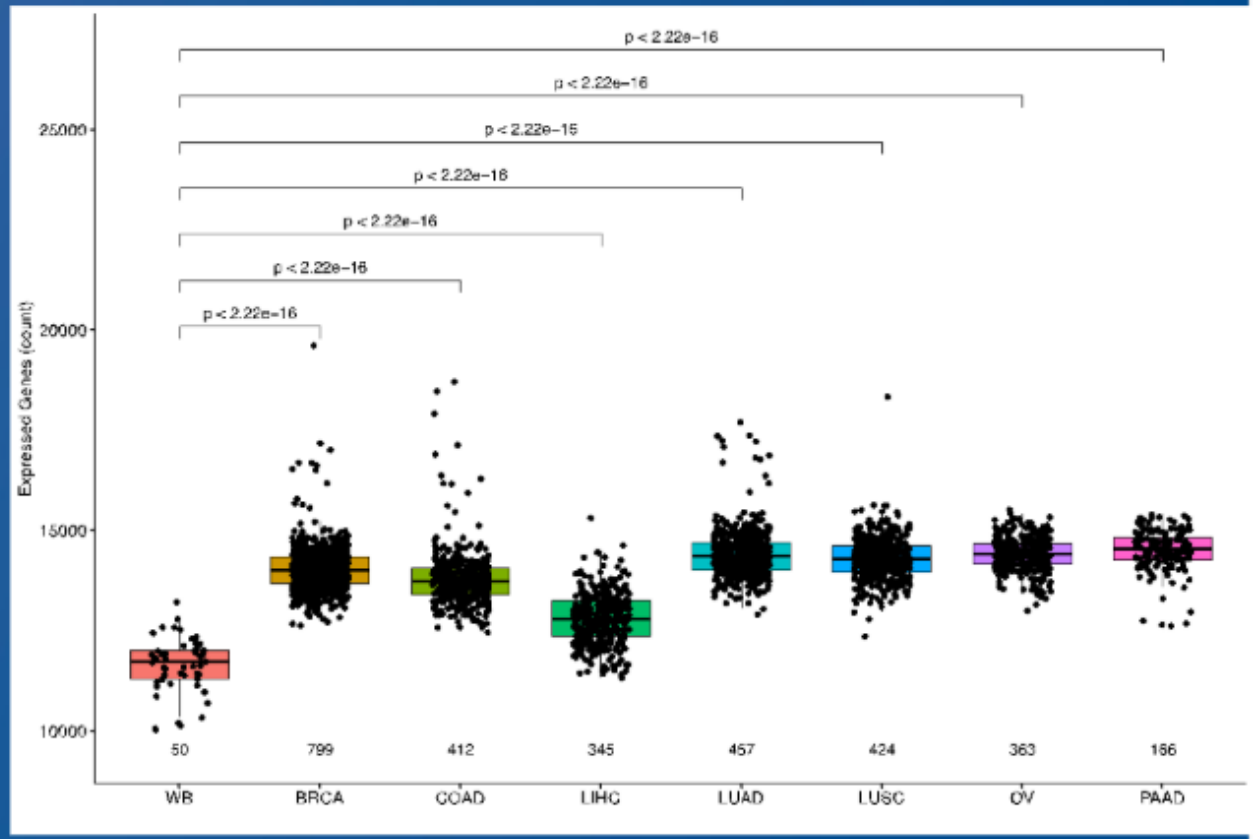


Methylation



Gene expression

Conclusions

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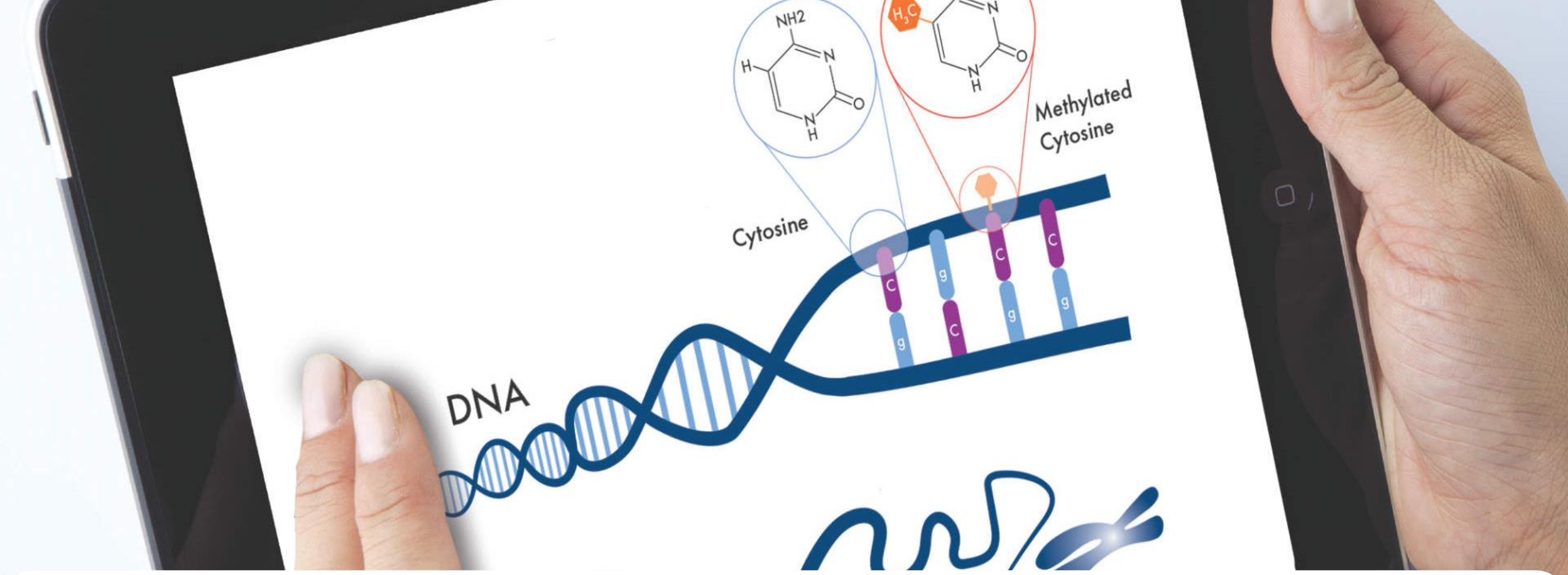
- Methylation impacts cfDNA fragmentation and results in different motifs around the ends of cfDNA-fragments
- Methylation-specific differences in fragmentation can be used to sensitively detect cancer and further improve CNV-based methods
- Requires data on differentially methylated CpGs in the cancer-type
- Increased cancer-specific signals overcome TCP-based limits
- This method fits into a set of analyses on lcWGS of cfDNA (1x – 2x)



QIAseq Targeted Methyl Panels

A liquid biopsy-compatible solution for detection of methyl markers using NGS

Hélène Bauby, Senior Global Product Manager, Targeted Genomics and Enterprise Genomics Solutions

ELBS Workshop, March 26th, 2025

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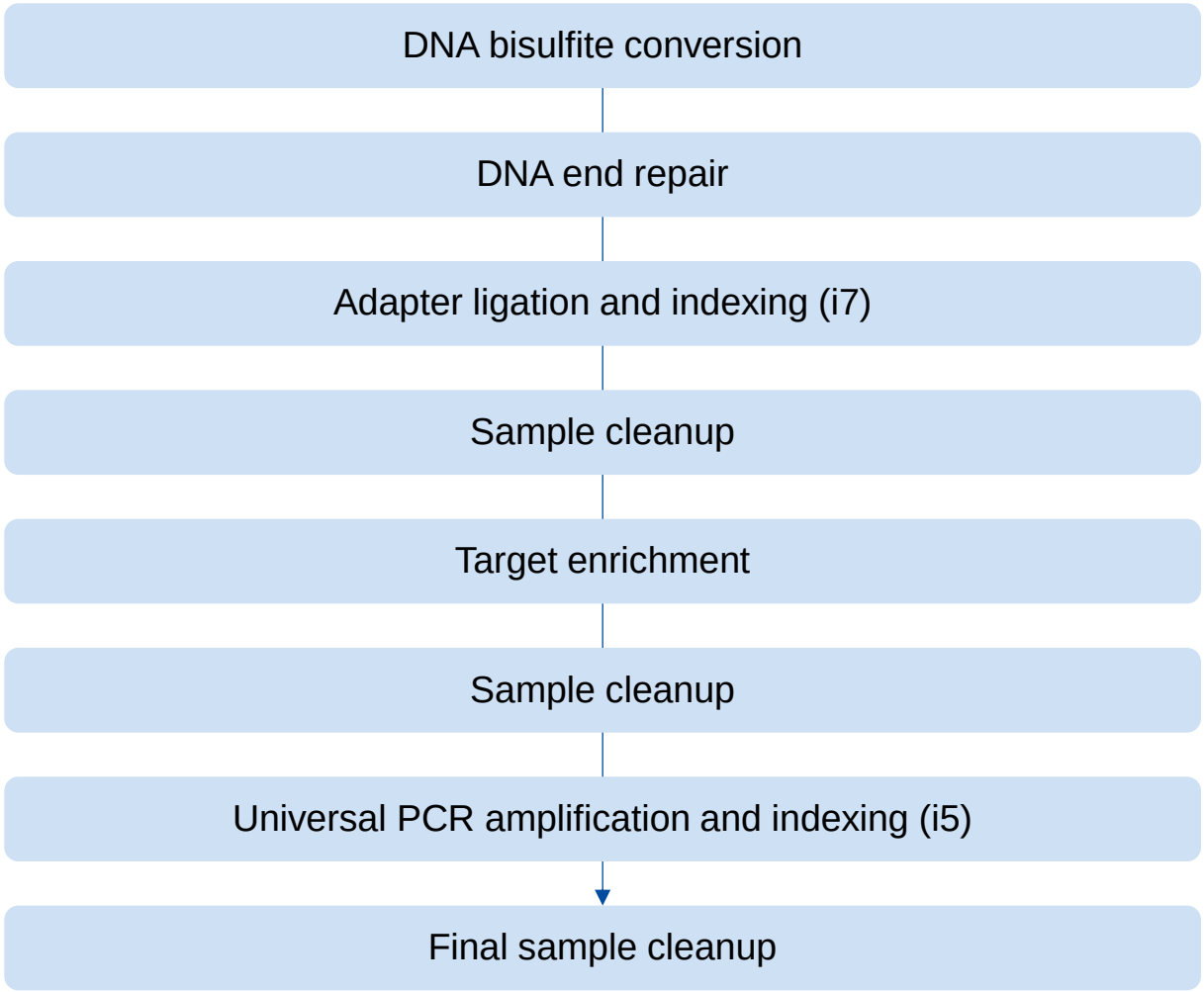
QIAseq Targeted Methyl Panels



- Also compatible with DNA enzymatic conversion
- QIAseq Enrichment Technology
- One day workflow with fast processing
- Optimized chemistry for great efficiency

QIAseq solutions for methylation detection

Panel box (kit)

- Proprietary PCR chemistry to enrich even GC-rich regions
- Optimized bisulfite conversion with the DNA Protect buffer
- Primers based on QIAseq target enrichment technology for enhanced uniformity and ability to detect both known and novel fusions
- Off-the-shelf and custom panels

Index box (kit)

- UMI-containing library adapters to incorporate UMIs

Digital Insights

- UMI-aware data analysis pipelines
  - Methylation events

Methylation profiling

QIAseq Targeted Methyl Panels – Catalog Panels

1. Human Breast Cancer Panel; MHS-001Z
2. Human Colorectal Cancer Panel, MHS-002Z
3. Immuno-Oncology Panel; MHS-201Z
4. Human T-cell Infiltration Panel; MHS-202Z

QIAseq Targeted Methyl Panels

Customize your panel with GeneGlobe or Enterprise Genomics Solutions

A Sample to Insight workflow for Methylation analysis on cfDNA from urine or blood

- QIAcube Connect
- EZ2 Connect
- QIAsymphony

QIAxcel Connect

NGS Liquid Handler

QIAxcel Connect

Sequencers

Local or cloud-based analysis

cfDNA QC

Library QC

Sample stabilization

Cell-free DNA stabilization in blood or urine samples

- PAXgene Urine Liquid Biopsy Set
- PAXgene Urine Liquid Biopsy Tube
- PAXgene Blood ccfDNA Tubes*

Sample preparation

- QIAamp Circulating Nucleic Acid Kit
- QIAsymphony Circulating DNA Kit
- QIAsymphony PAXgene Blood ccfDNA Kit

Bisulfite treatment and Library preparation

- EpiTect Fast DNA Bisulfite Conversion Kits
- **QIAseq Targeted Methyl Panels and index kits**

NGS run

- Illumina
- Element
- Other sequencers with an added conversion step

Data analysis

- QIAGEN CLC Genomics Workbench (Local)
- GeneGlobe Data Analysis Center

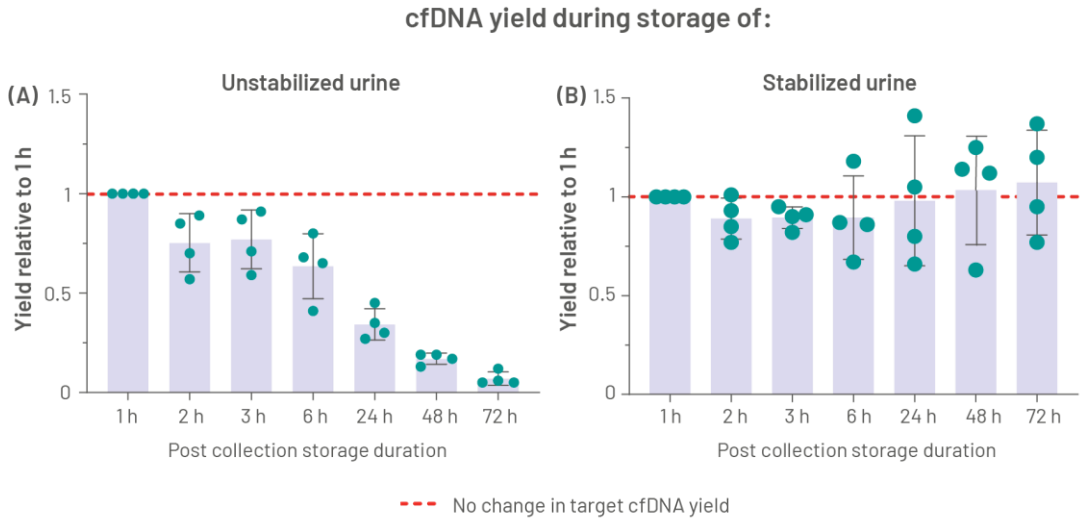
Targeted methylation using cfDNA from urine samples

PAXgene Urine Liquid Biopsy Set (RUO*)

The cfDNA profile in urine is **impacted post-collection** during storage by

- cfDNA degradation
- genomic DNA release from body cells
- bacterial growth

Which is minimized with the **PAXgene Urine Liquid Biopsy Set**



This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No. 824110.

More details on product webpage, available on www.qiagen.com and www.preanalytix.com

*The PAXgene Urine Liquid Biopsy Set (RUO) is intended for Research use only.
Not for use in diagnostic procedures.

Designed to be a **user-friendly** urine collection device that enables **convenient urine collection** for individuals and laboratory professionals

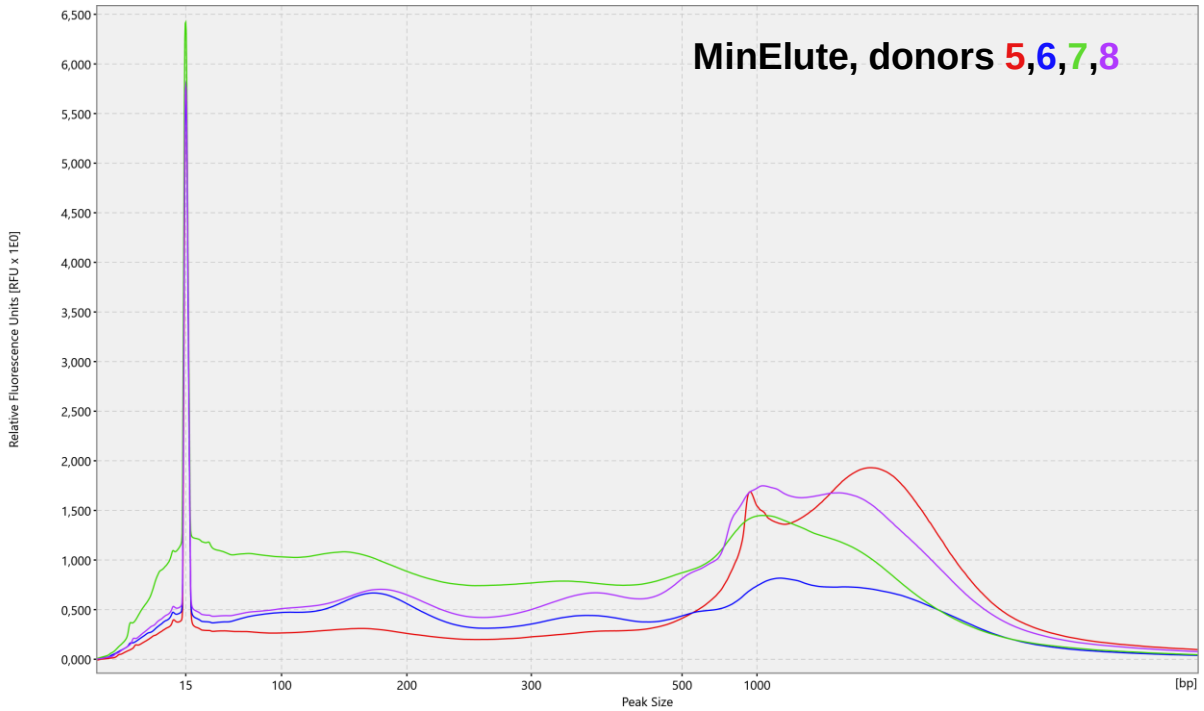
Enables **closed sample transfer** into **a sterile tube** minimizing **contamination** and biospecimen **exposure**

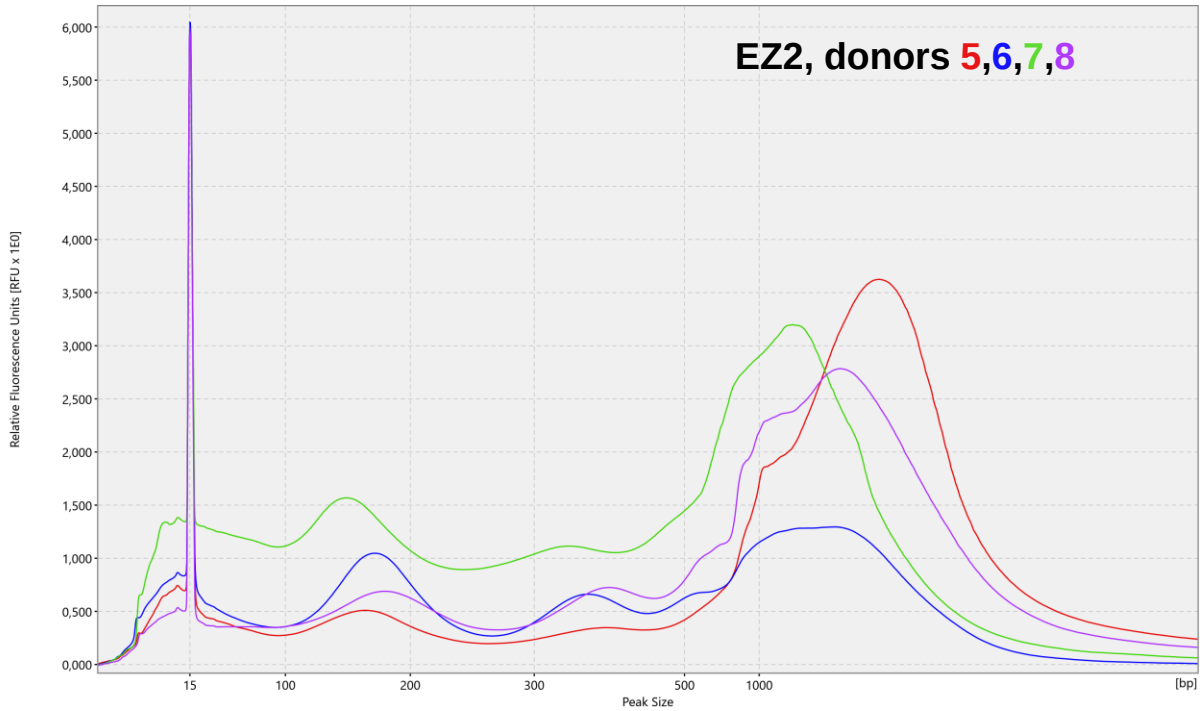
Enables a consistent **sample to additive ratio** allowing **standardized** sample processing **without crosslinking.**

Included in a complete and **optimized preanalytical workflow** for urine cfDNA analysis.

QIAxcel Connect can be used for the quality control of cfDNA samples

- cfDNA (11–18 ng), as quantified by Qubit, in 6 µL on QIAxcel DNA High Sensitivity Cartridge
- Signal in mono- and dinucleosomal area; smaller and much bigger fragments are also visible

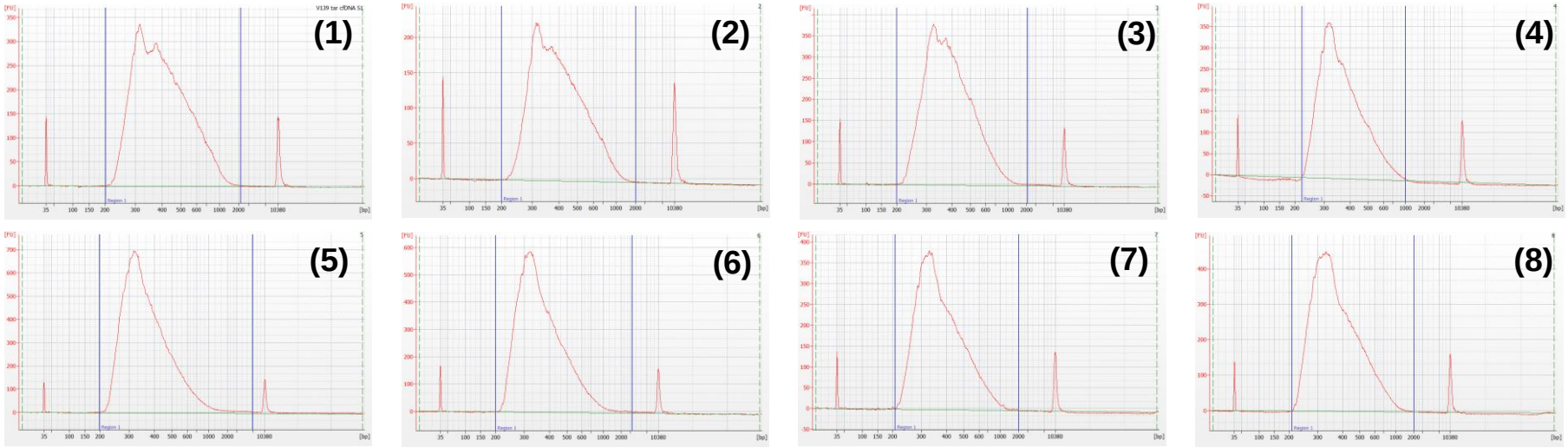




QIAseq Targeted Methyl Panels generate libraries from urine cfDNA samples

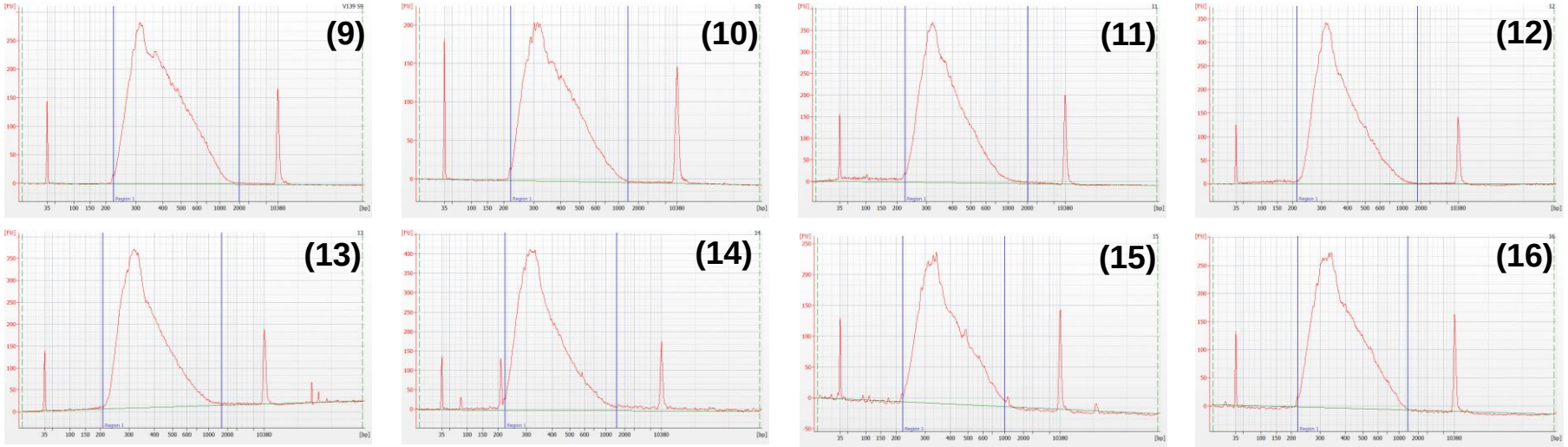
QIAamp MinElute

- 50 ng cfDNA
- Purification using 1.2x QIAseq Beads

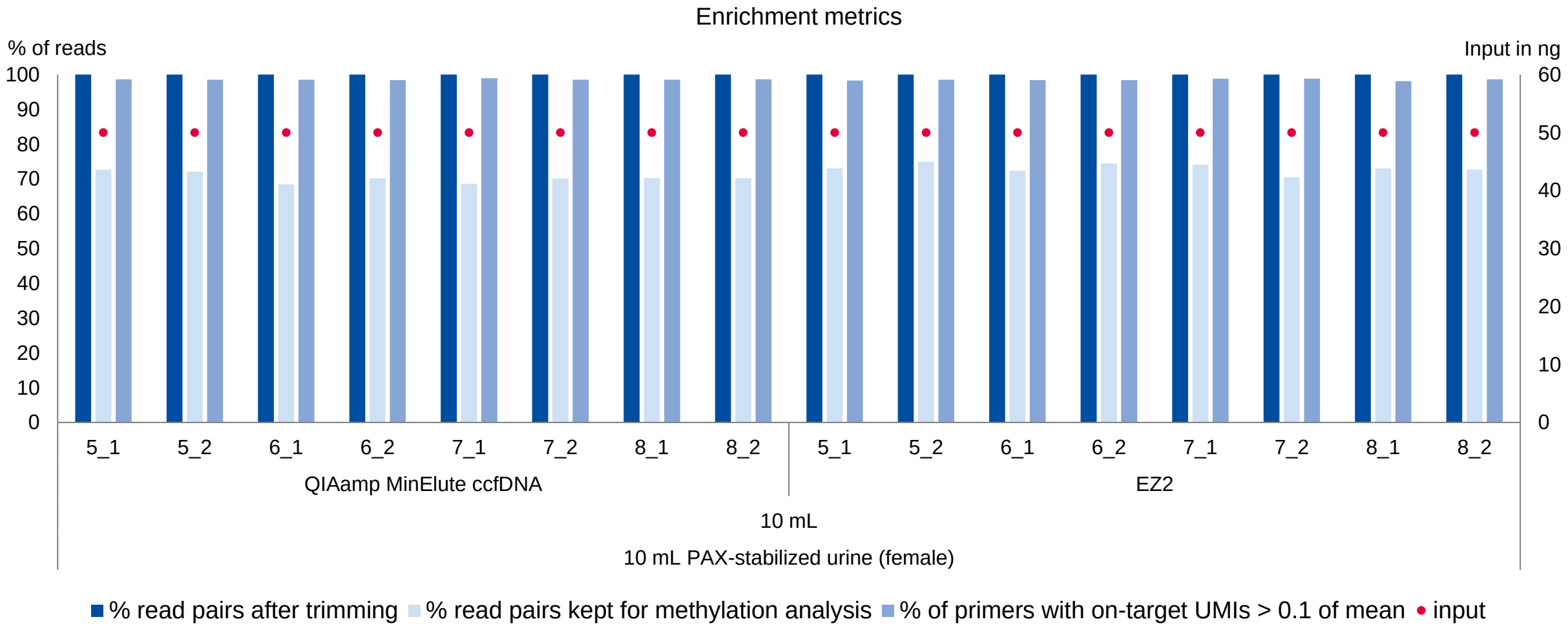


EZ2

- 50 ng cfDNA
- Purification using 1.2x QIAseq Beads

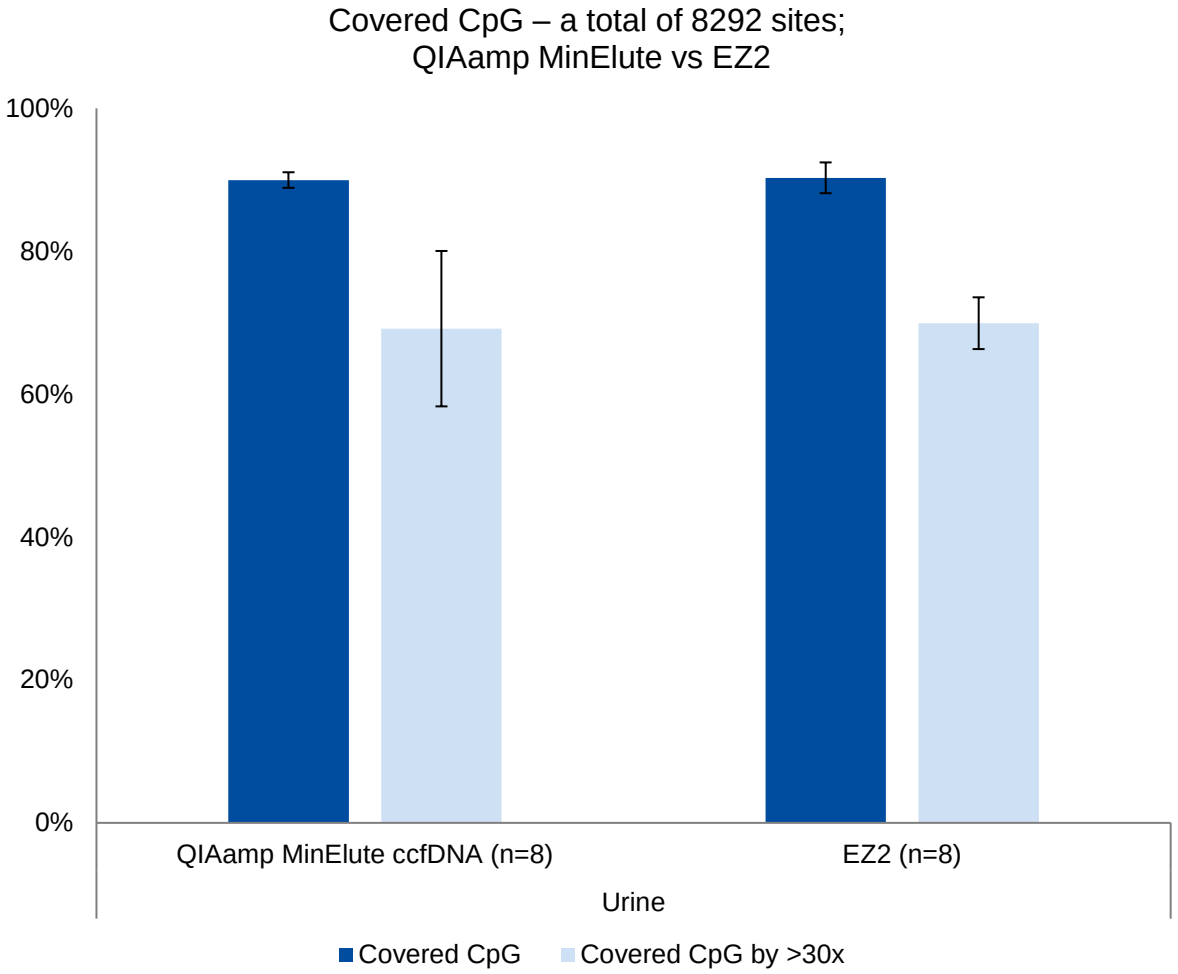


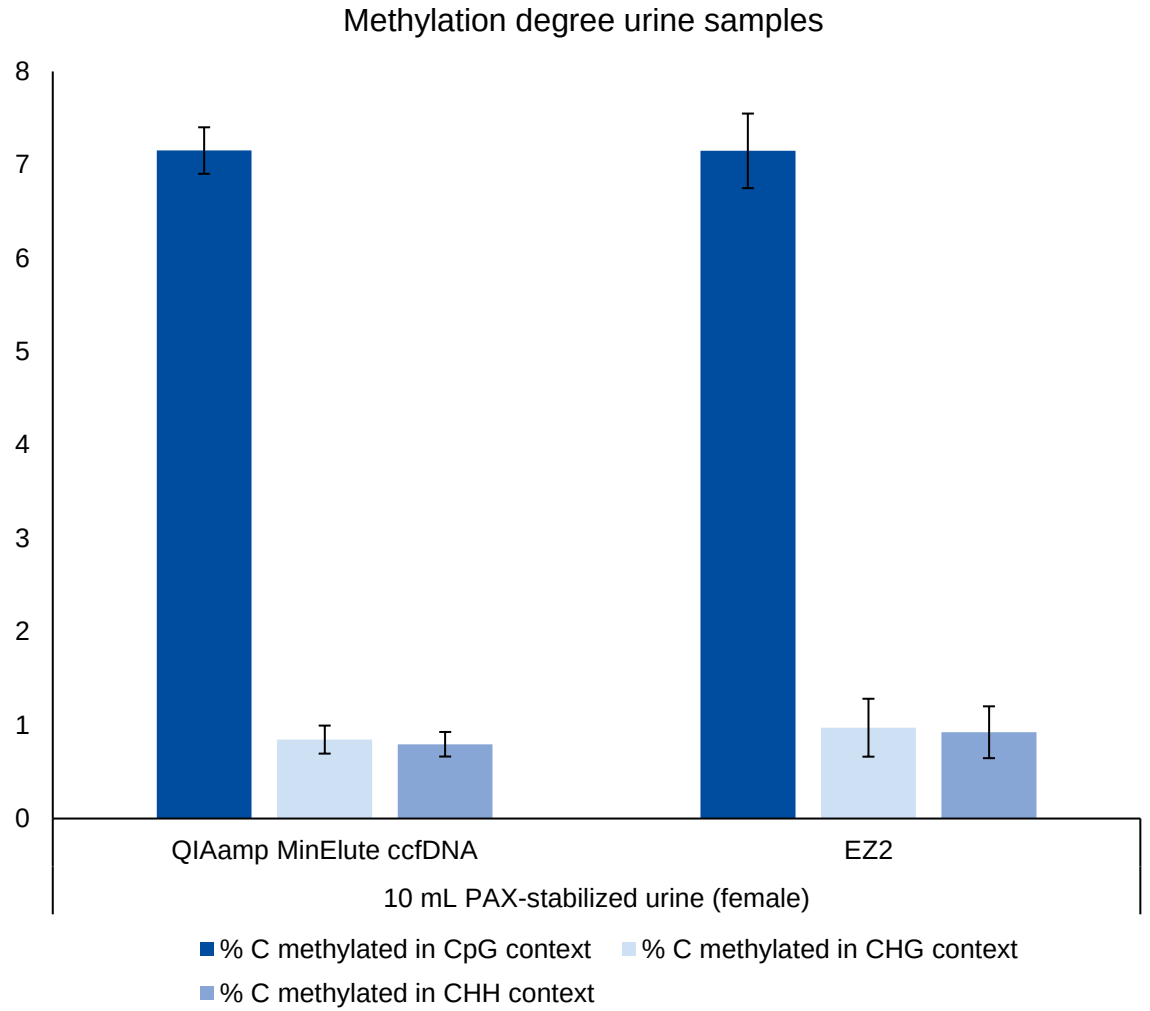
QIAseq Targeted Methyl Panels enable the generation of high reproducible and high-quality libraries_MiSeq



Sequencing: V2, PE 2x150bp on Miseq run with an Average of 860K reads per sample; 10% PhiX spike in

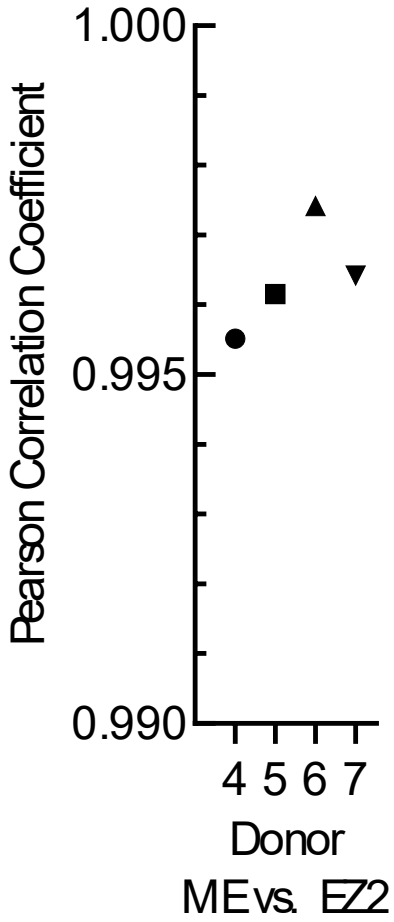
Detection of the methylated ratio – CpG statistics

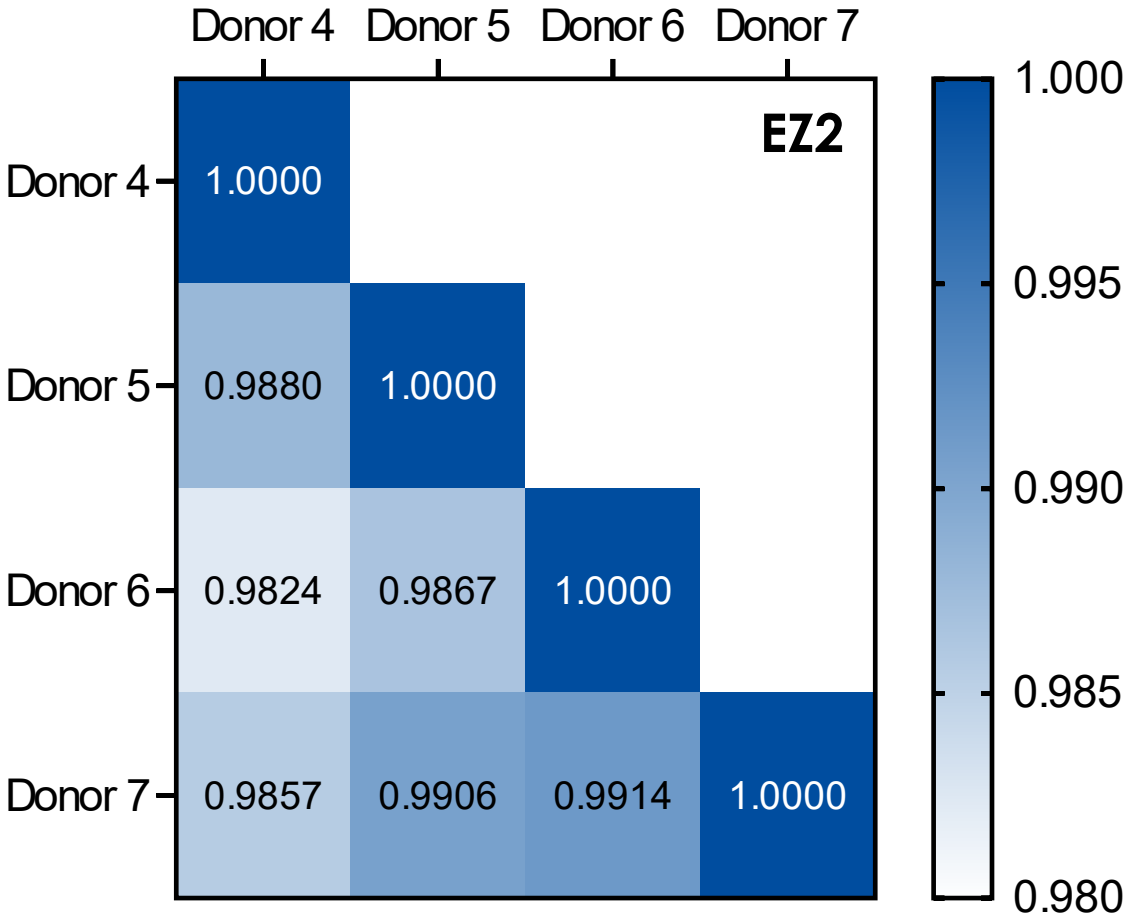




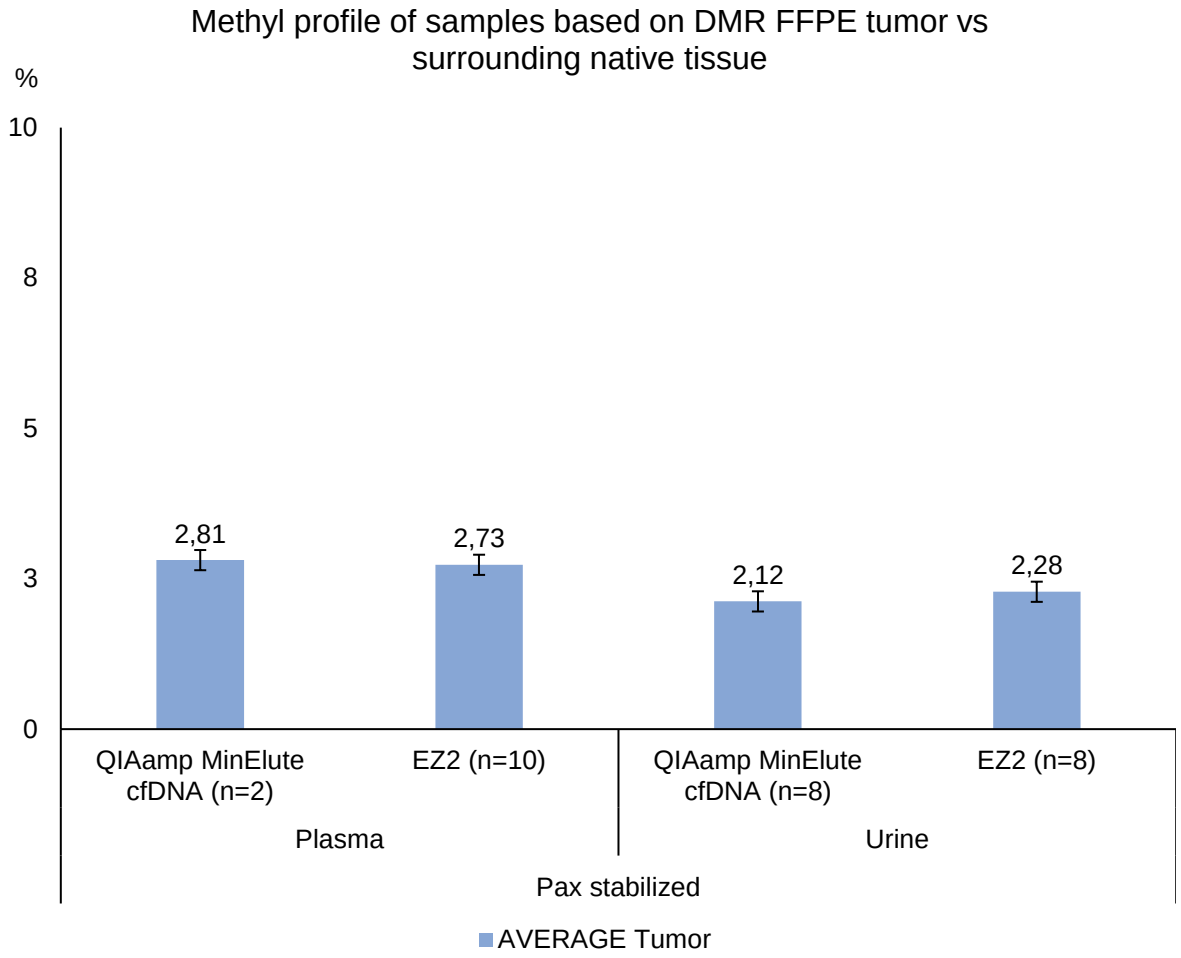
AVRG reads/UMI = 2.1

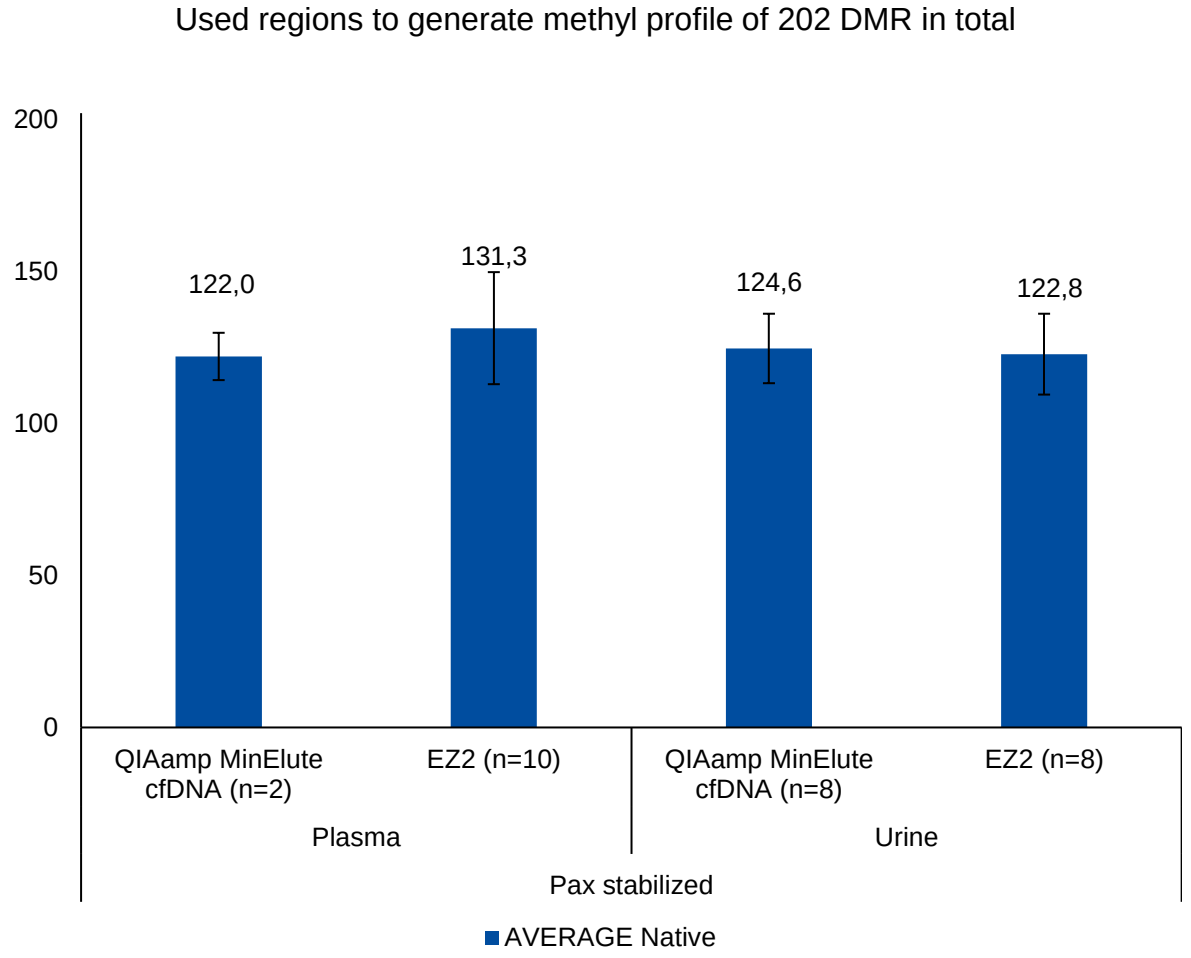
QIAseq Targeted Methyl Panel show good correlation between samples





Methyl profile based on DMR generated from urine samples from healthy donors



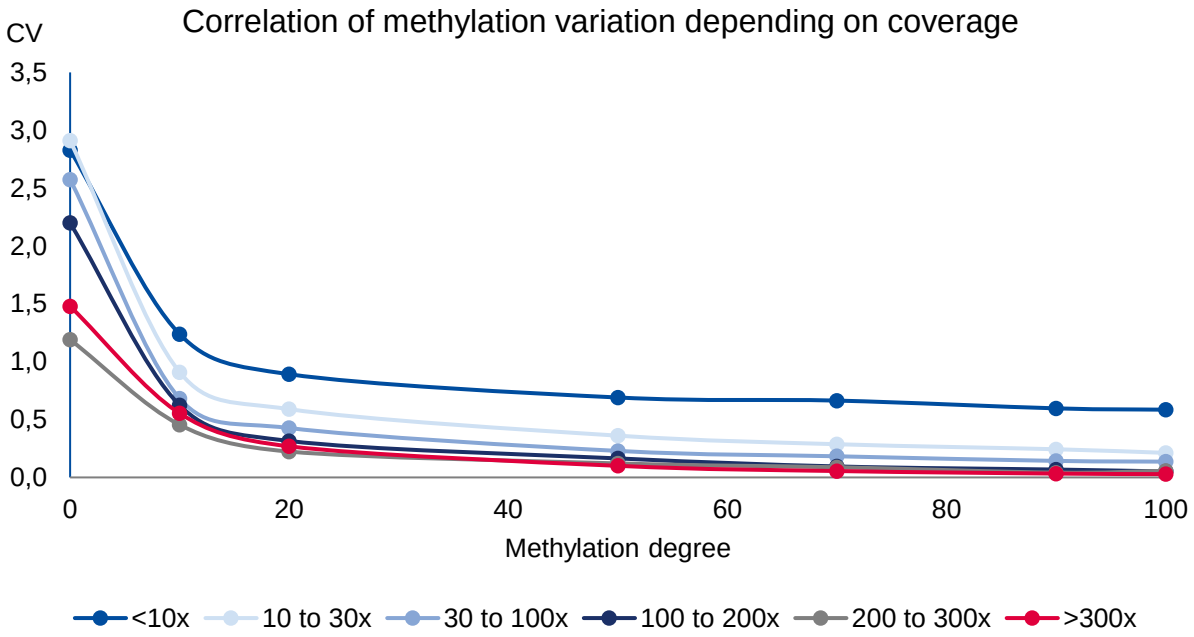


DMR analysis to generate the Data-base used in this profile analysis was generated using methylation comparison between CRC-FFPE sample vs Native tissue.

Accuracy of methylation degree evaluation vs coverage

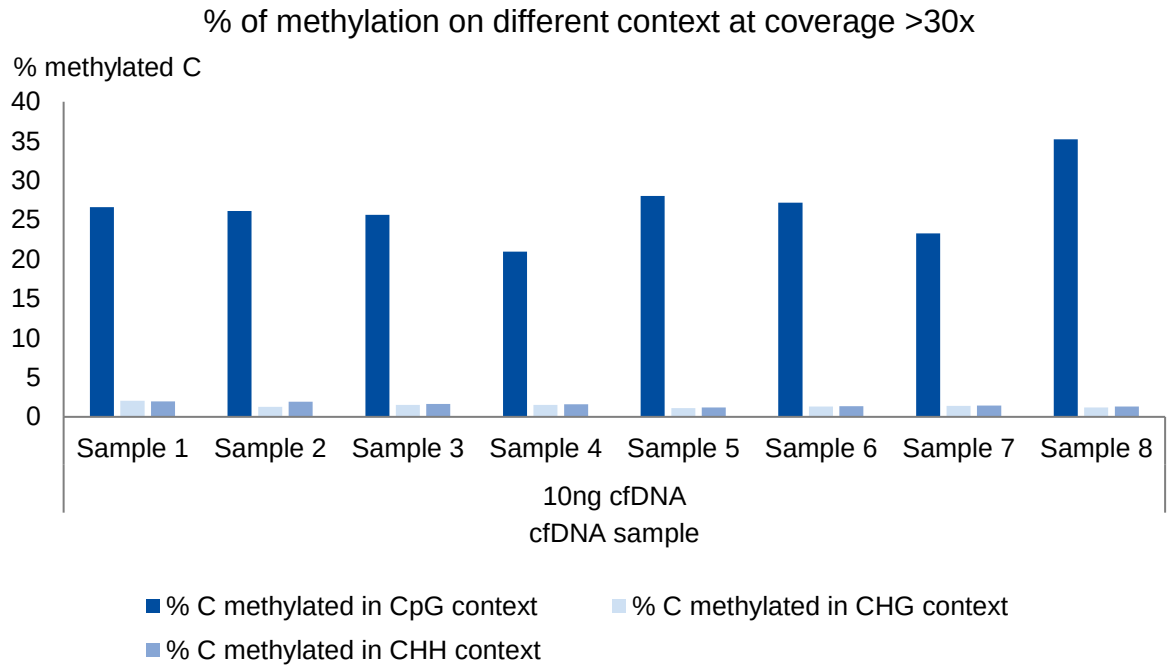
Higher methylated regions are accurately called at lower coverage as lower methylated regions

- The plot represent the CV of called methylation degree at different depths represented by UMI-context reads (<10x 10-30x, 30-100x, 100-200x, 200-300x and >300x)
- A coverage of >30x will give a good overview of the hyper and hypomethylated status, whereas >300x will accurately call also low methylation degree



The plot represent average methylation degree of CpG and non CpG Sites of different cfDNA samples

- Methylation on CHH and CHG contexts can be used to monitor conversion efficiency as it is described to be unmethylated in mammalian cells



Thank you for your attention. Questions?

Advancing donor-derived circulating tumor methylated DNA reference standards

Krystyna Nahlik

Current Biosynthetic Methylation Reference Standards

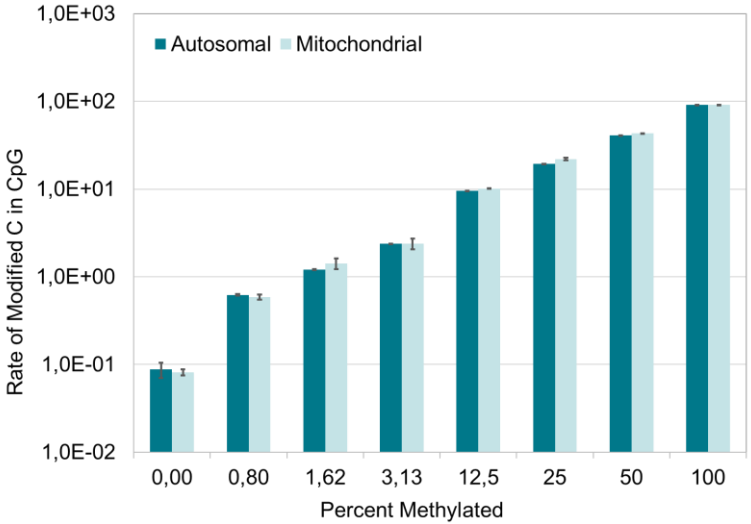
Methylation assay targets are often not disclosed (the Epi proColon™ SEPTIN9 amplicon is an exception; Cologuard™ looks at NDRG4 and BMP3; ColoSure™ looked at VIM).

Seraseq Methylated ctDNA Mutation Mix is designed to address the general analytical validity of a methylation assay, irrespective of targets.

- Derived from GM24385 cell line gDNA to utilize a well-characterized genome
- Two separate vials: **~0 %** CpG methylation / **~100 %** CpG methylation
- Customers can blend these as needed to determine if they observe the expected level of genome-wide CpG methylation

Evaluated in NIST manuscript “Development, characterization, and inter-laboratory validation of methylated human cell free DNA candidate reference materials” (He *et al,* in preparation)

Methylation reference materials enable precise assay performance evaluation



Rate of CpG site modification in autosomal and mitochondrial chromosomes in the umethylated and methylated ctDNA mixes combined at various levels, measured by biomodal duet solution +modC bioinformatics pipeline.

% MetC	Sensitivity (%)	Specificity (%)
0.00	97.69	99.96
0.80	97.85	99.91
1.62	97.78	99.88
3.13	97.68	99.90
12.5	97.86	99.91
25	97.86	99.89
50	97.61	99.88
100	97.79	99.92

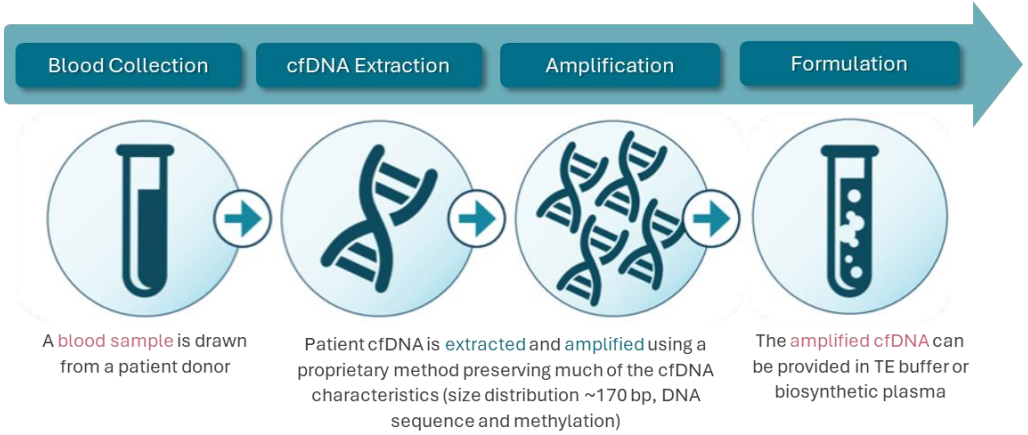
Sensitivity and specificity of CpG site modification measurement by the biomodal duet solution calculated using the methylated and unmethylated ctDNA mixes.

LGC CLINICAL DIAGNOSTICS

Higgs *et al.*, presented at AACR 2024

New Donor-derived Reference Standards

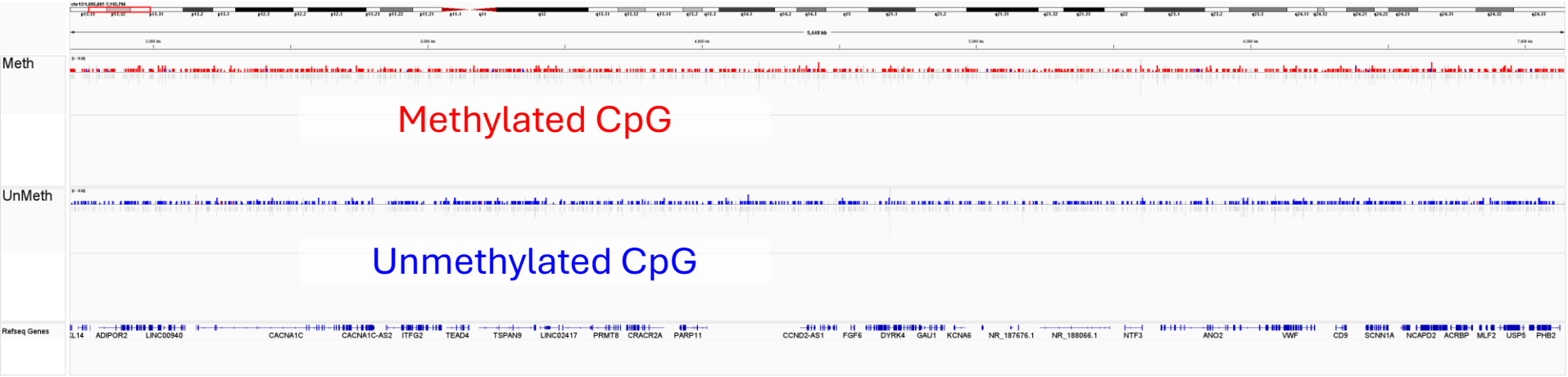
- Designed to mimic patient samples; reproduce patient ccfDNA fragments, mutations, and CpG-island methylation status, could be used to assess analytical validity for specific tumor types
- Produced using a method similar to one used for Seraseq donor-derived NIPT reference materials
- Prototype based on a CRC patient ccfDNA, the same approach can be used to create donor-derived standards from different tumor types and stages

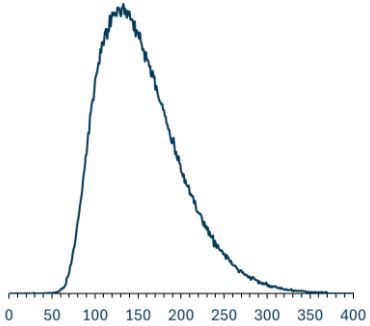


We can also make methylation standards with the current method using patient ccfDNA as the starting material:

- Patient-specific mutation status
- Genome-wide 0% or 100% CpG methylation

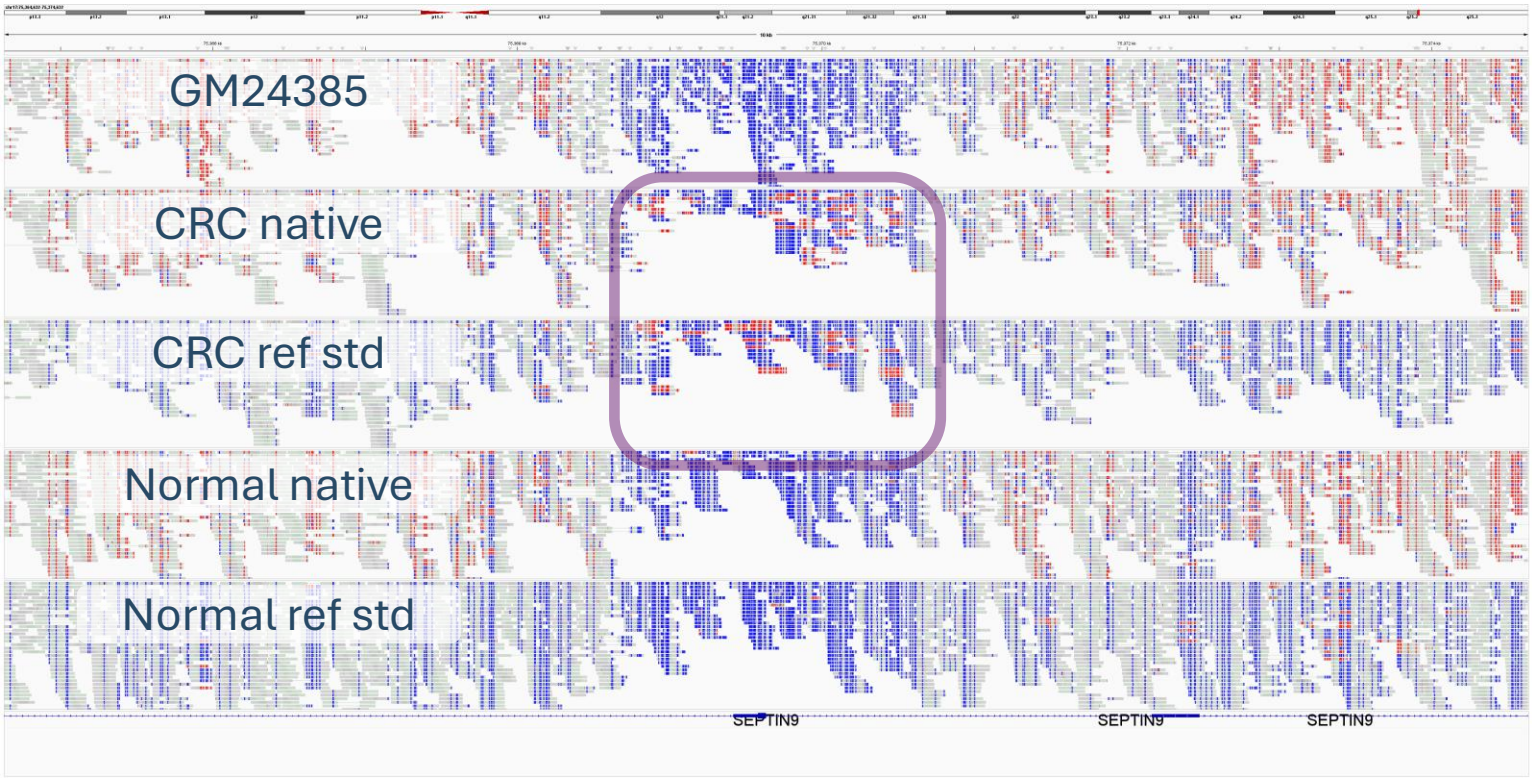
Low-pass EM-seq of the current methylation standards

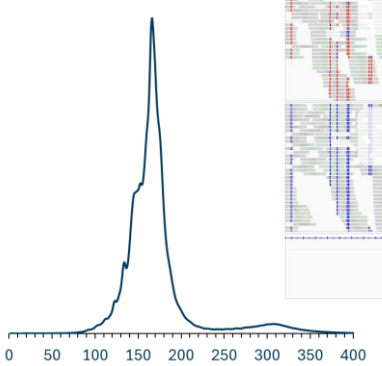




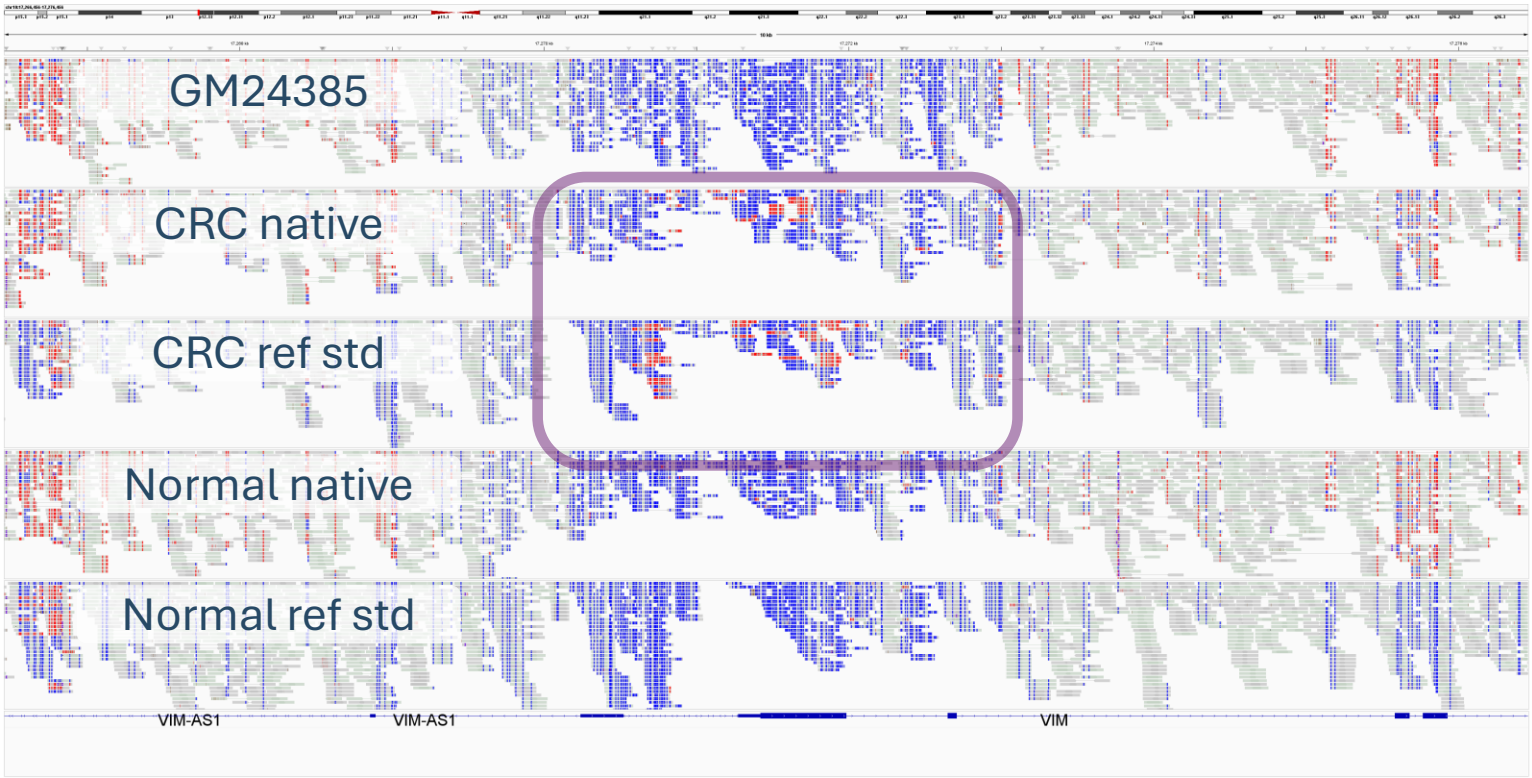
This and following slides: internal LGC data, courtesy of Y. Konigshofer

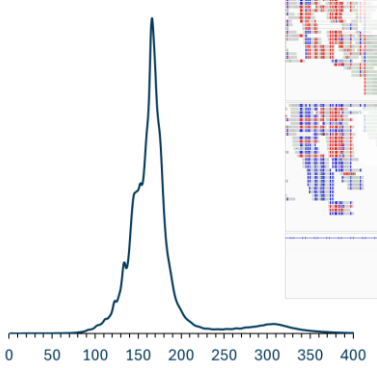
EM-seq of Donor-derived Standards – SEPTIN9



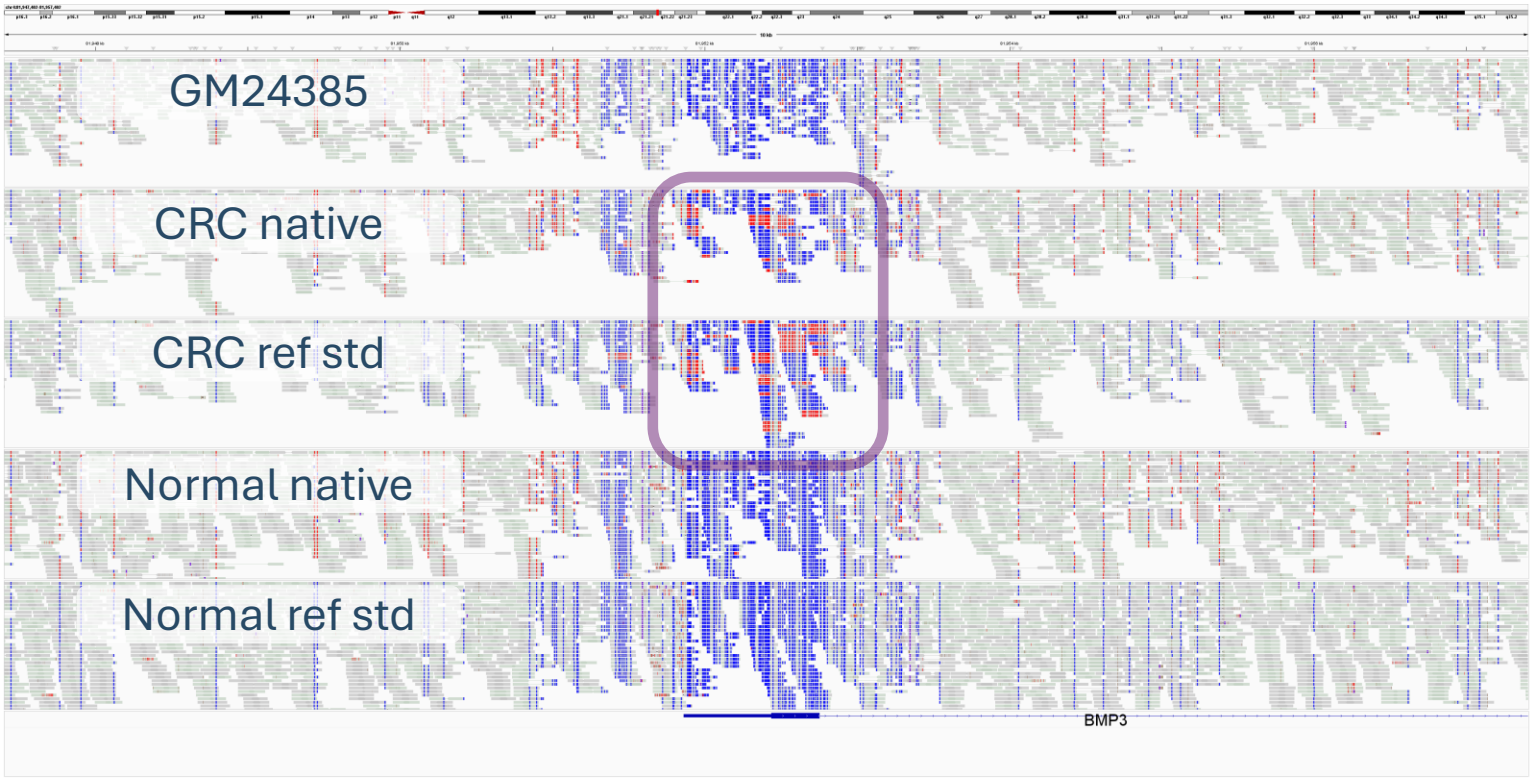


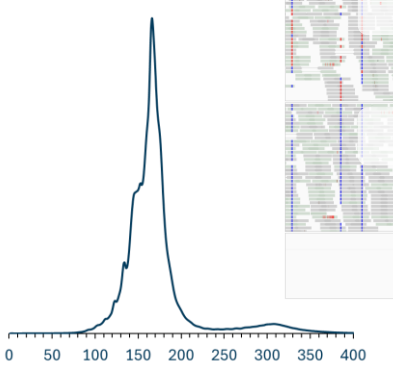
EM-seq of Donor-derived Standards – VIM



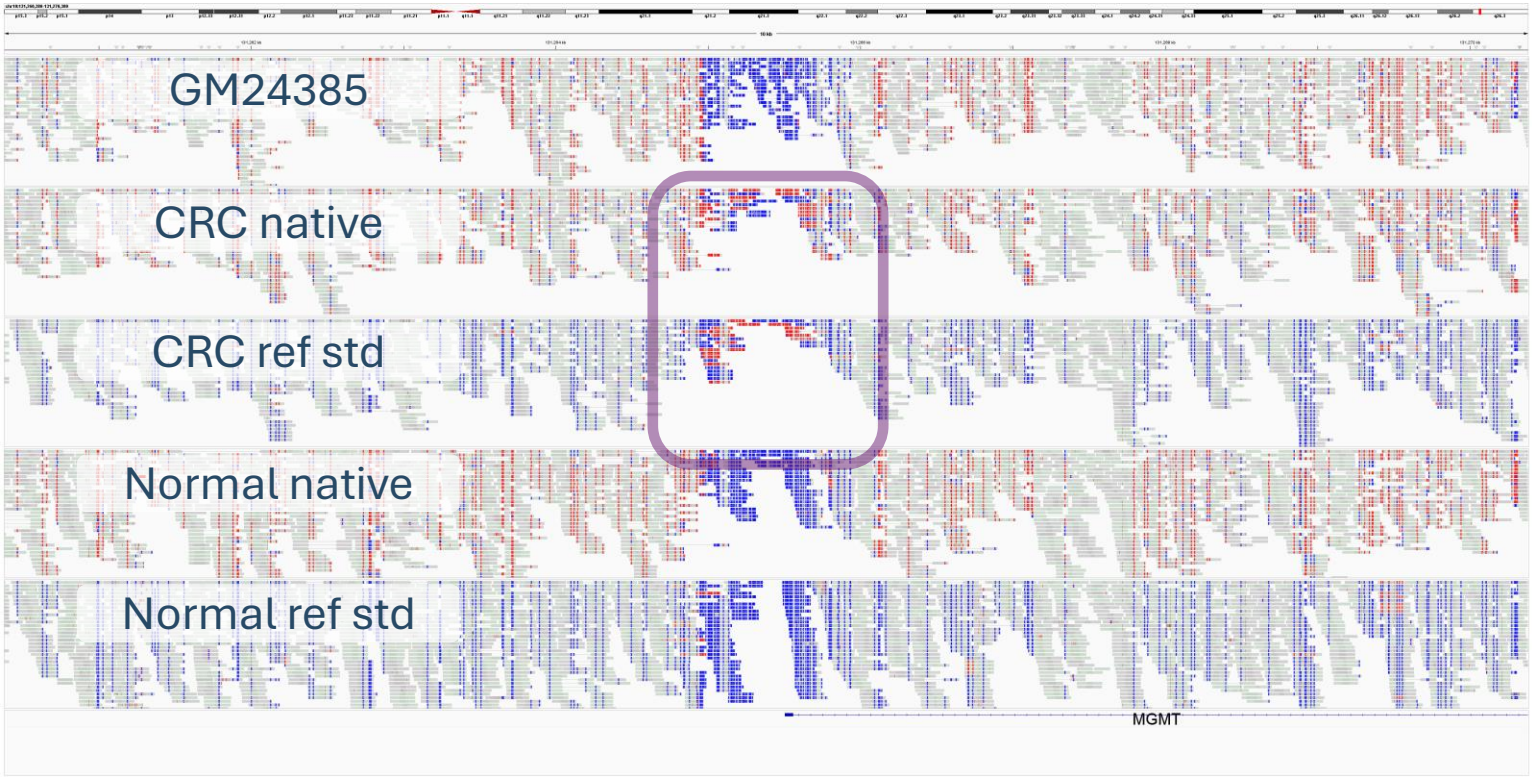


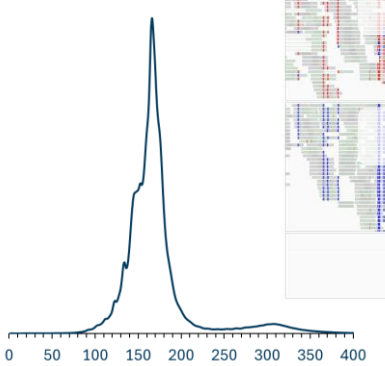
EM-seq of Donor-derived Standards – BMP3



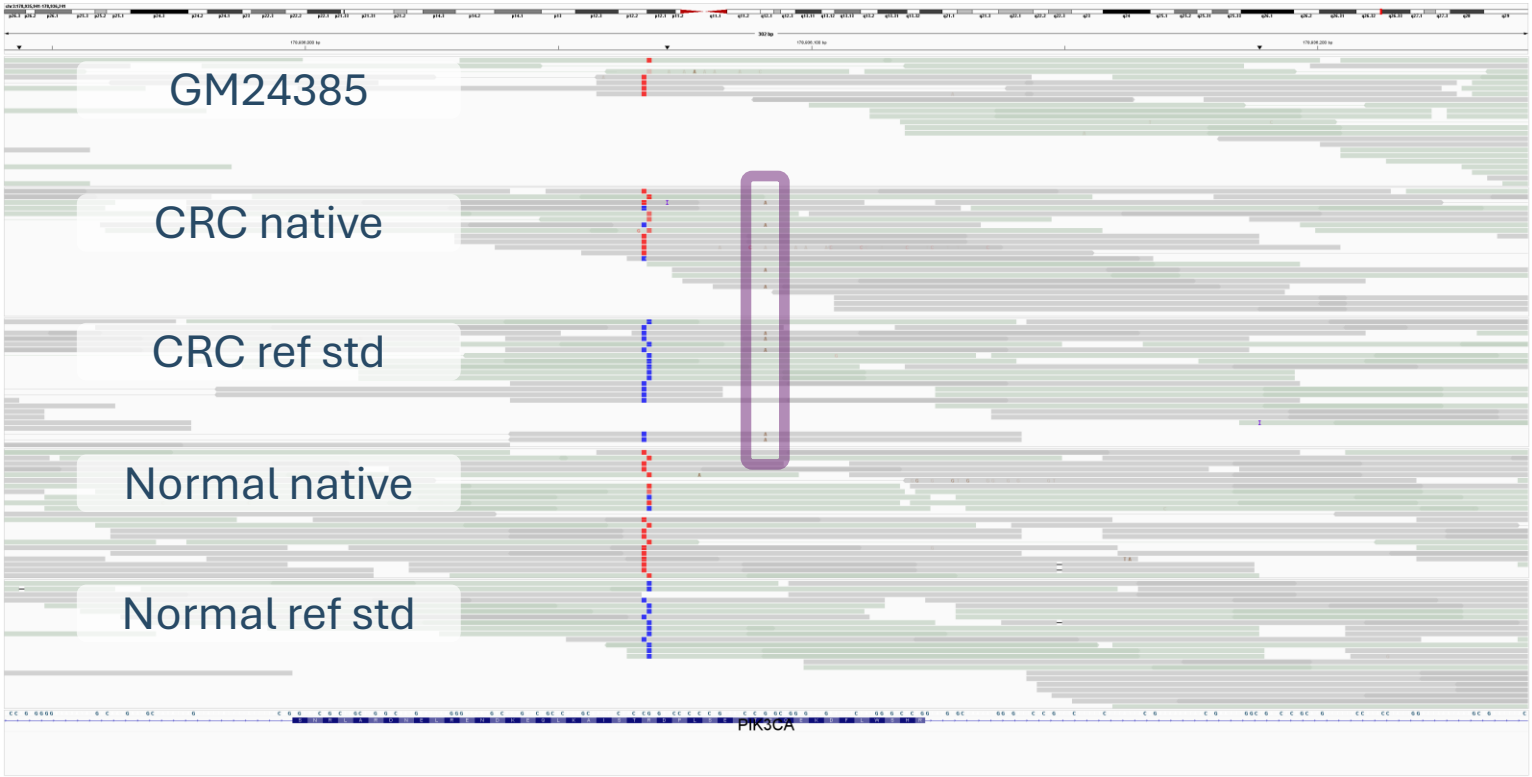


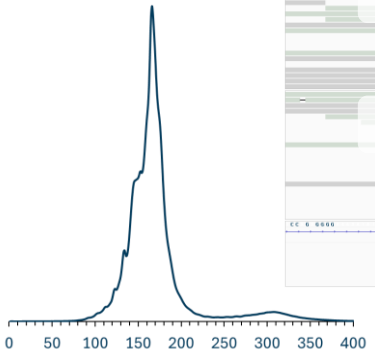
EM-seq of Donor-derived Standards – MGMT



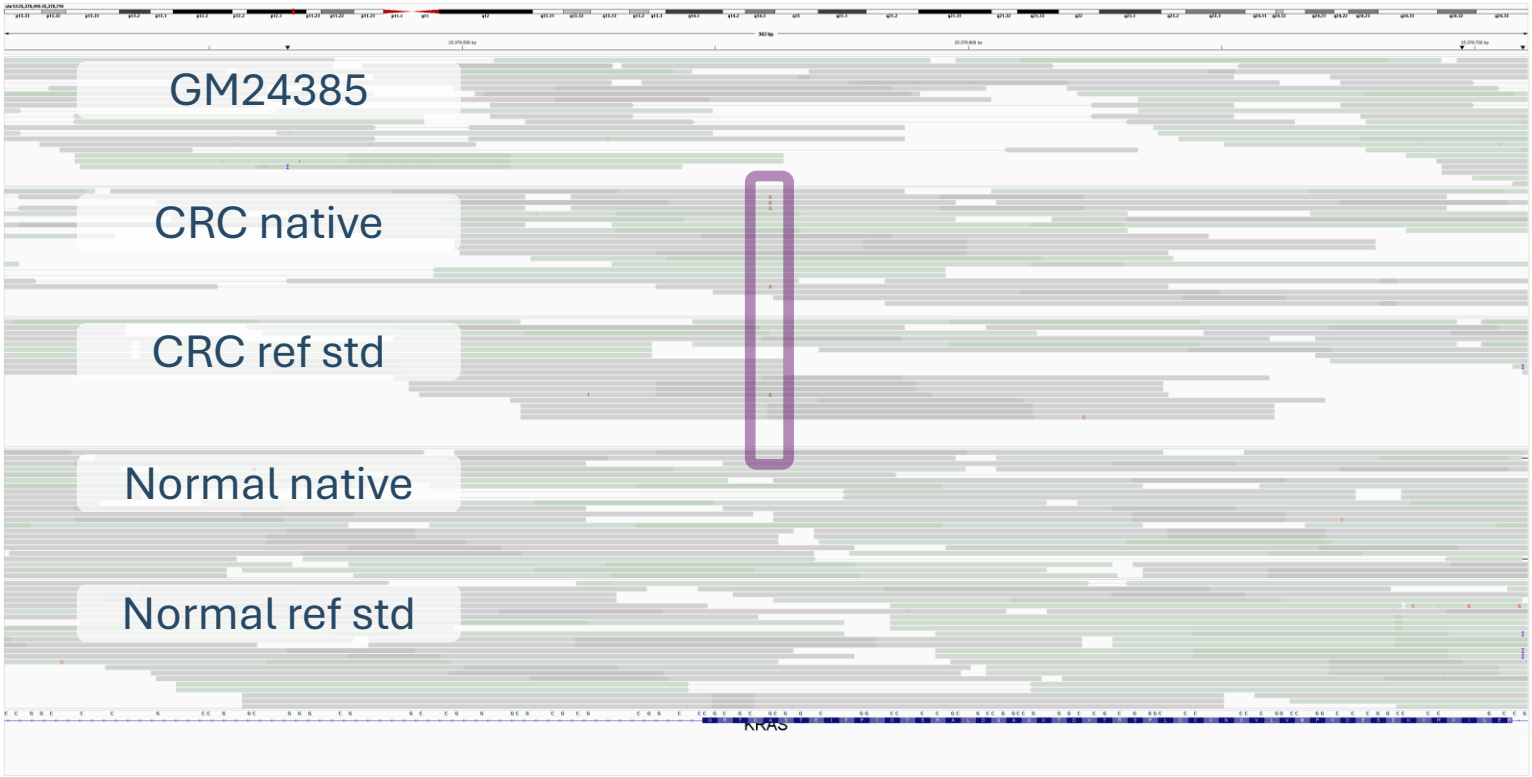


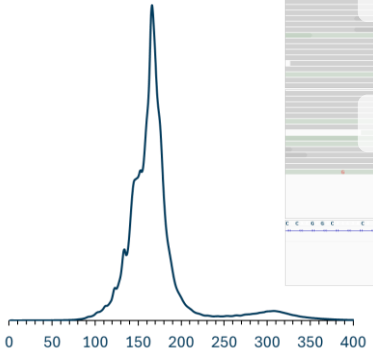
WGS of Donor-derived Standards – 24 % PIK3CA E545K



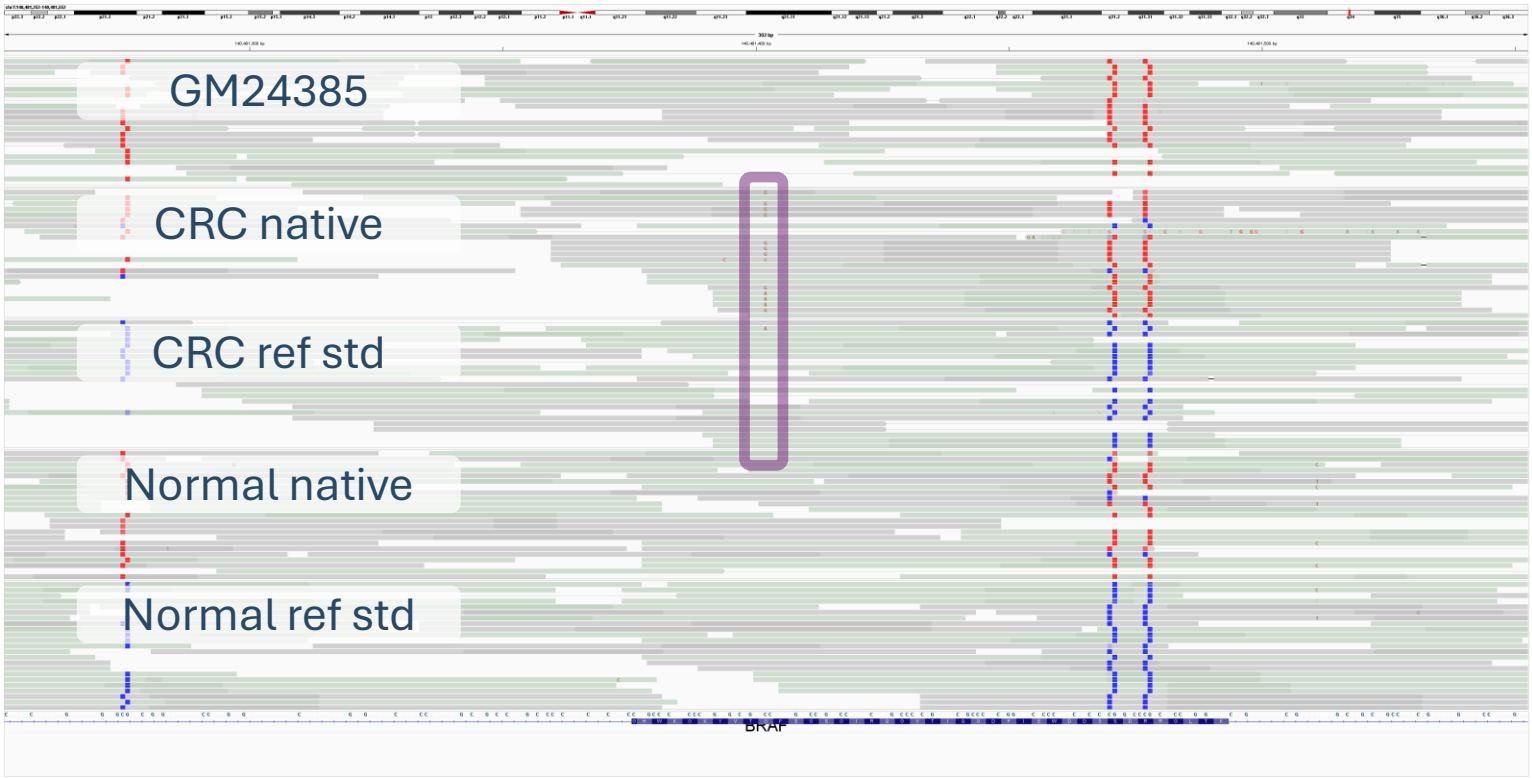


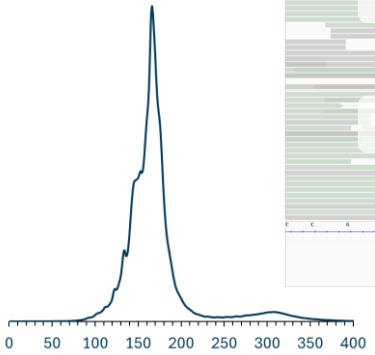
WGS of Donor-derived Standards – 14 % KRAS A146V





WGS of Donor-derived Standards – 12 % BRAF G469A





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