Hybrid capture based sequencing library approach for cell free DNA from urine of colorectal cancer patients

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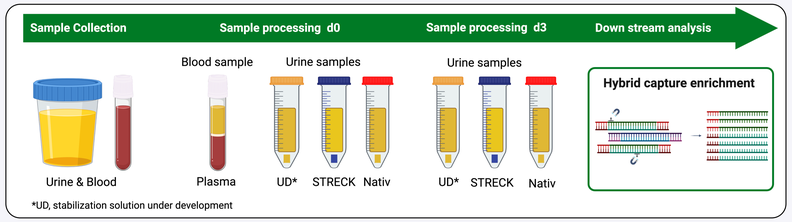
1 Background

The study of body fluids other than blood is gaining increasing attention in the field of liquid biopsy. Urine is a particularly valuable specimen since it offers complementary information to blood and is a truly non-invasive sampling method. Evidence suggests that urine cell free DNA (ucfDNA) harbors information about renal and bladder cancer. However, not much is known about ucfDNA in colorectal cancer (CRC) patients. Therefore, we performed a feasbilility study of a hybrid capture based cfDNA NGS approach including the optimization of the preanalytical workflow for CRC urine.

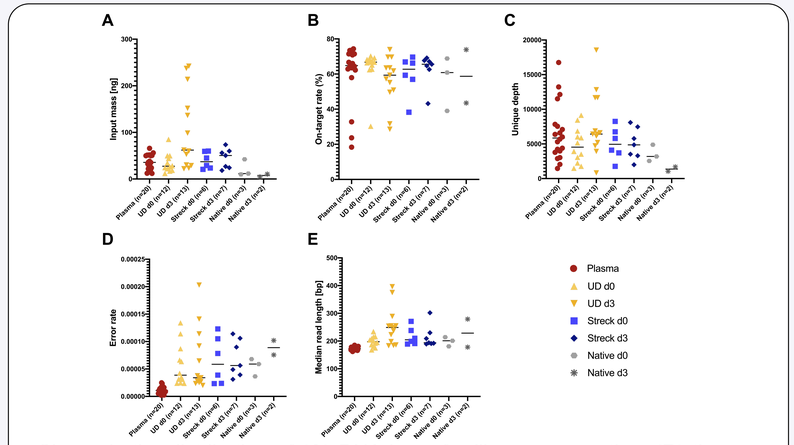
2 Research study set-up



- In this research study urine specimen were collected from 20 patients with metastatic CRC, aliqouted and stored with or without a stabilizing agents at room temperature.Urine collection/ stabilization solution under development, provided by PreAnalytiX (QIAGEN/BD company) for the CD Laboratory project, and Streck Urine Preserve were used for stabilization.
- Urine cfDNA was isolated at the day of donation (d0) and after 3 days (d3) using the QIAsymphony platform (QIAGEN).
- Matched blood samples were additionally collected in PAXgene Blood ccfDNA Tubes (PreAnalytiX).
- cfDNA samples were analyzed using the AVENIO ctDNA Analysis Kit (Roche), a hybrid-capture based approach enriching for 17 clinically relevant genes.

Disclaimer: This work was performed in the framework of a Christian Doppler Laboratory, which is established at the Medical University of Graz, with non-university partner PreAnalytiX. All products used in this study are RUO products. The reference to patients refers to the source of clinical samples used for scientific insight. No diagnostic or therapeutic action was taken based on the research findings in this study.

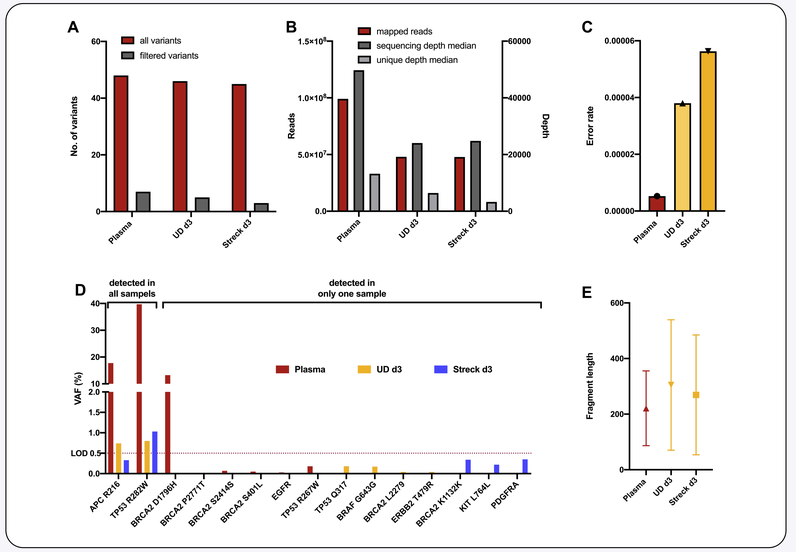
3 Hybrid capture based sequencing is feasible for ucfDNA



Shown is the distribution of **(A)** DNA input for library preparation, **(B)** the on-target rate, **(C)** the average unique sequencing depth, 6047 reads for plasma and 4889 reads for urine samples, **(D)** the error rate, **(E)** and the fragment length.

- While all stabilized ucfDNA yielded high quality libraries, library preparation failed in 66.7% (data not shown) of nativ urine samples, demonstrating that cfDNA rapidly degrades after collection if it remains unstabilized.
- In nativ samples in which sequencing data could be obtained, unique sequencing depth was significantly decreased compared to stabilized samples.
- For stabilized samples, sequence analysis revealed full concordance between urine and plasma for putative germline variants (data not shown).
- If low ucfDNA (less than 50 ng) input was available, a low signal-to-noise ratio with a high number of false positive “low level” variants was observed.
- We therefore adjusted the limit of detection for ucfDNA to 0.5%, at which CRC-related mutations were detected in ucfDNA in one patient. Although these variants, were not observed in plasma cfDNA, they could be detected in all stabilized urine samples, which indicates true variants (see (4)).

4 “Tumor derived variants” can be detected in urine of mCRC patients



- As a representative case, one patient is shown of which plasma, UD (at d0 and d3) and Streck Urine Preserve (d3) was available.
- **(A)** Number of variants before and after filtering for germline variants and variants < VAF 0.5%.
- **(B)** Number of reads and sequencing depth for cfDNA extracted from plasma and urine.
- **(C)** Error rates were higher in urine which led to a higher sequencing background compared to plasma cfDNA.
- **(D)** Plasma cfDNA sequencing identified three mutations in *APC*, *BRCA2*, and *TP53* with variant allele frequencies of 17%, 13% and 39%, respectively. The *APC* and *TP53* mutation could also be found in urine samples.
- **(E)** Representation of fragment length in plasma (average 169 bp, range 138–204), and in urine (average 234 bp, range 168–396).

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CONCLUSION: ctDNA could be detected in 14/20 (70%) of plasma samples, of which only 3 patients had tumor fractions of >5%. The detection rate in urine cfDNA was low, with only one patient with concordant variants above the detection threshold. As higher urine volumes should easily be assessable in most cases, a higher amount of ucfDNA could be extracted to address this point. Nevertheless, our data demonstrate that a hybrid-capture based analysis approach is feasible for ucfDNA. Due to degradation and lower concentrations, immediate stabilization of urine after donation is required. Moreover, the limit of detection needs to be adjusted if low amounts of input DNA are available.

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