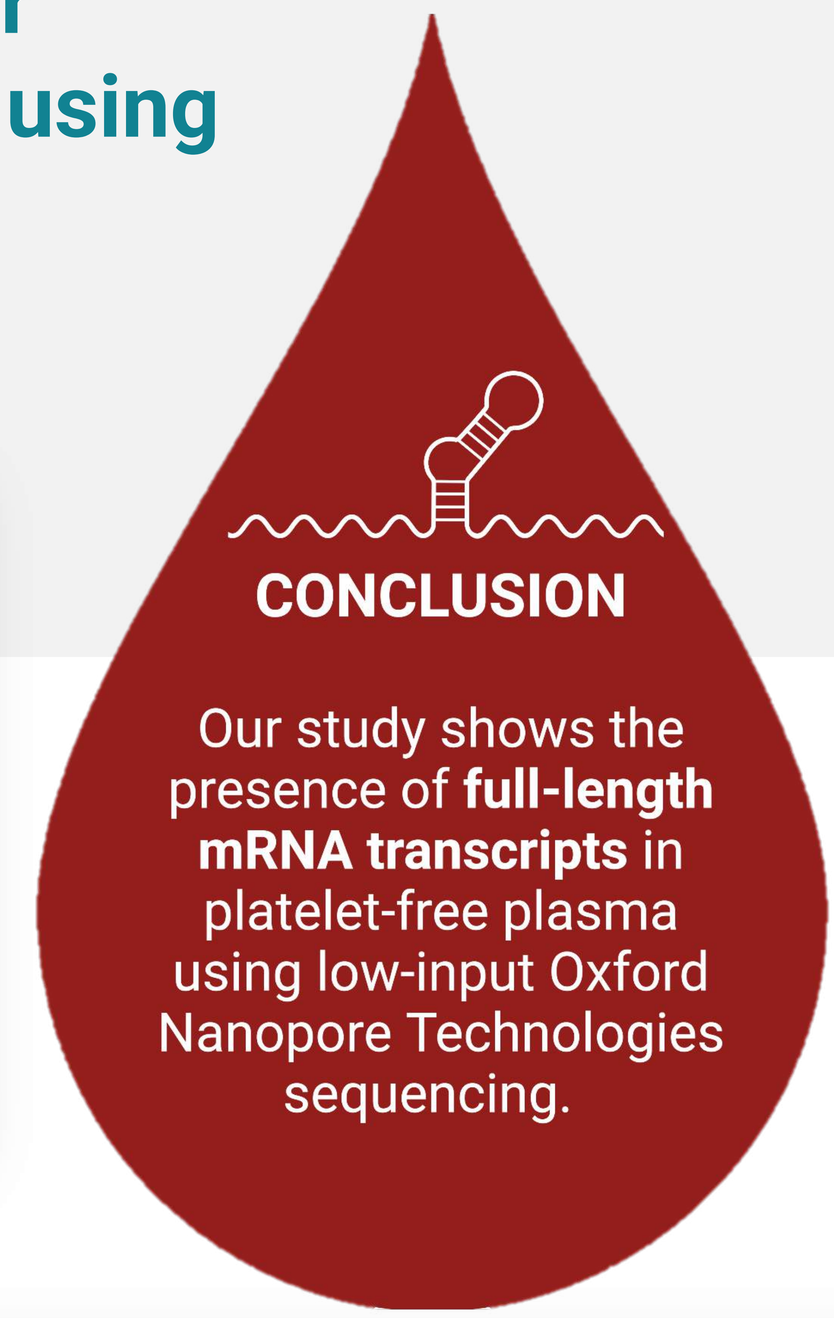
Uncovering the full-length extracellular transcriptome in human blood plasma using long read cDNA sequencing

Jasper Verwilt, Pieter Mestdagh, Jo Vandesompele

BACKGROUND & AIM

Does full-length mRNA exist in blood plasma?

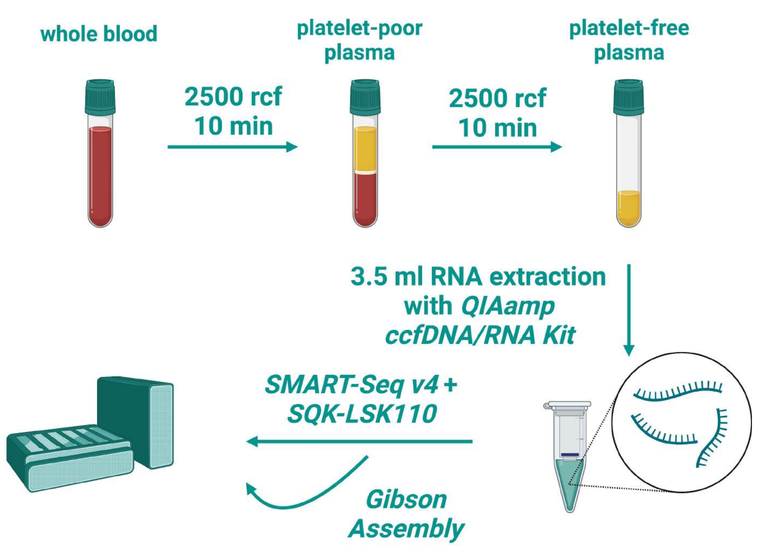
Due to the recent rise in transcriptome sequencing of liquid biopsies, the extracellular transcriptome in human plasma is slowly but steadily sharing its secrets. Most RNA sequencing in human plasma is performed using short-read sequencing technology. Given their intrinsic limitations, the fragmentation status of the plasma RNA has remained elusive. To date, full-length RNA sequencing data of human plasma is not available, and the current thinking is that all extracellular RNA in platelet-free plasma is highly degraded.



For the first time ever, we sequenced plasma mRNA using Oxford Nanopore Technologies

METHODS

Large volume RNA extraction from platelet-free plasma, followed by SMART-seq v4 and SQK-LSK110.



RESULTS

Full-length mRNA in platelet-free plasma

Compared to the spike-in RNA, the plasma RNA is contains more reads around half of the original length of the transcript. Transcripts do exist with observed lengths close to their annotated length (Figure 1 and 2). Longer transcripts tend to be more fragmented, but shorter theoretical transcripts, the relationship fades (Figure 2). Hemoglobin transcripts represent the largest fraction of intact transcripts (Figure 3).

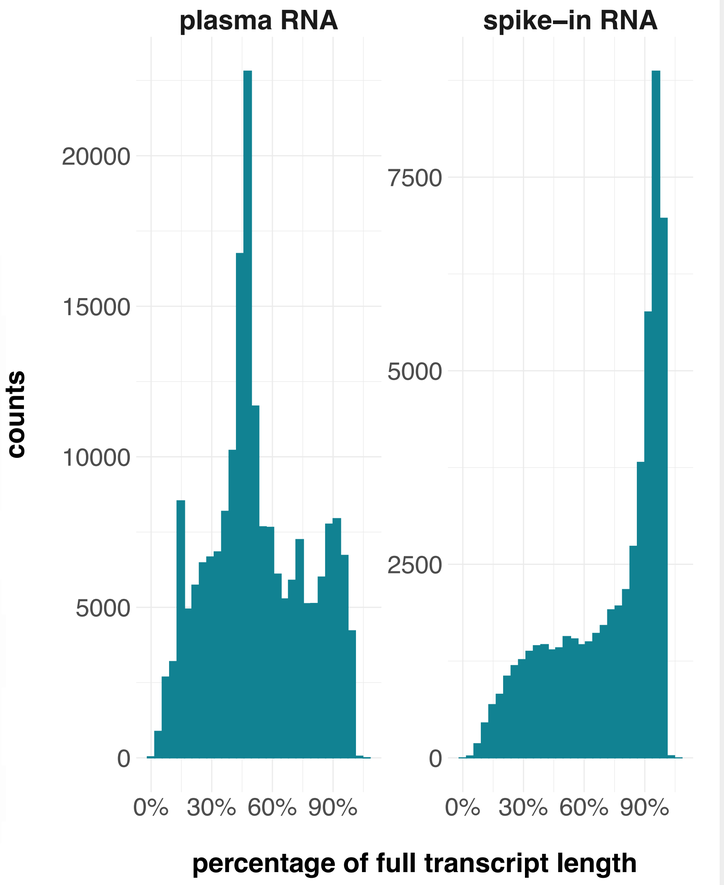


Figure 1: Histogram depicting the fraction of the length of the transcript and the theoretical, annotated length for RNA present in the plasma and spike-in RNA.

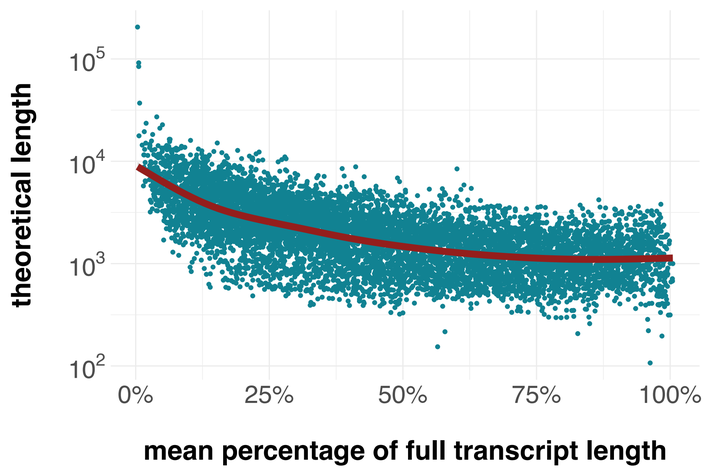


Figure 2: Dotplot showing the theoretical length of the transcripts in function of the per-gene average of the relative length of the fragment.

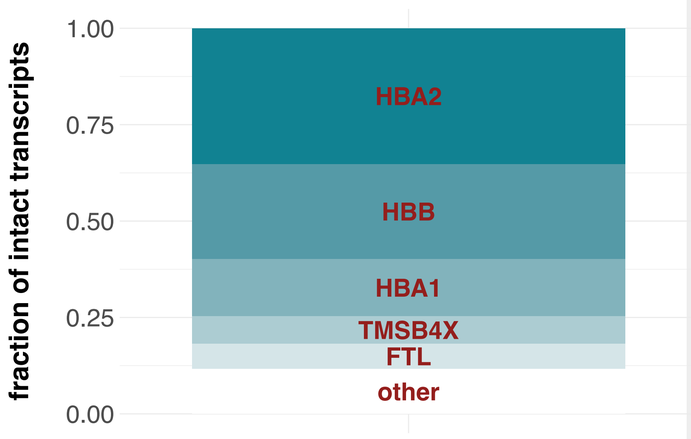


Figure 3: Histogram showing the five highest abundant transcripts with intact (>80% relative length) fragments. The remaining intact transcripts are depicted as "other".

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GET IN TOUCH!

Are you investigating RNA in liquid biopsies? I would love to know about your research and what you think of mine!

Jasper Verwilt

jasper.verwilt@ugent.be

@JVerwilt