Longitudinal gene expression from **dried blood microsamples**: a pilot study

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Introduction

Traditional venous blood collection for gene expression profiling and biomarker discovery requires a visit to a healthcare professional, posing challenges for special populations, large-scale studies, or at-home collection.

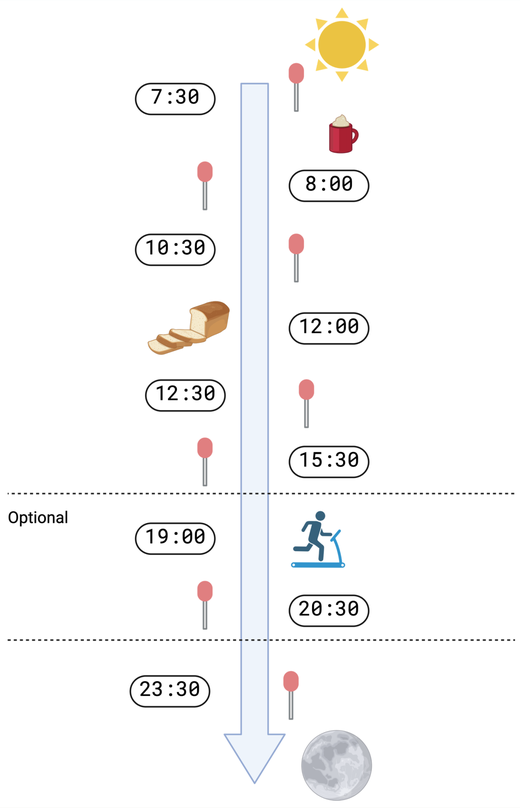
Capillary blood microsampling, the process of obtaining small volumes of capillary blood, can overcome these challenges.

Volumetric Adsorptive Microsample (VAMS) devices guarantee a constant volume of blood across donors and samplings.

Materials & Methods

1

12 healthy volunteers for self-collection of **7** blood microsamples on the same day following a strict schedule



2

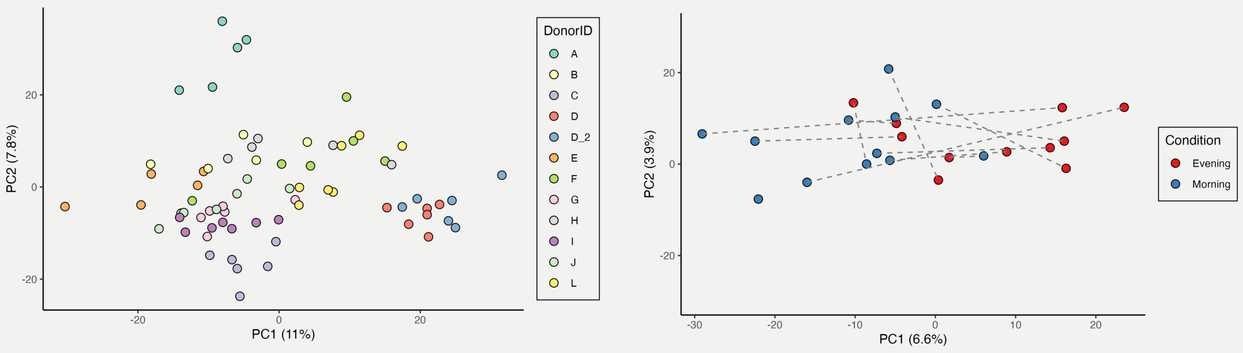
- **RNA was isolated** using the Maxwell RSC simplyRNA Blood Kit (Promega)
- **RNA integrity** was assessed using Fragment Analyzer (Agilent)
- Before cDNA synthesis, RNA was treated using **in-house designed globin mRNA blockers**. Library preparation was performed using the **QuantSeq Pool** kit (Lexogen)
- **Sequencing** on NextSeq 2000 platform (Illumina)

Results

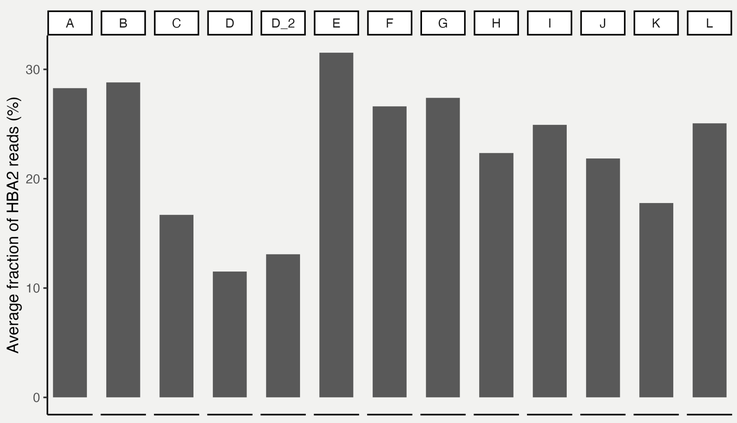
Exploratory analysis

A PCA before donor correction

B PCA after donor correction: 'evening' vs 'morning'

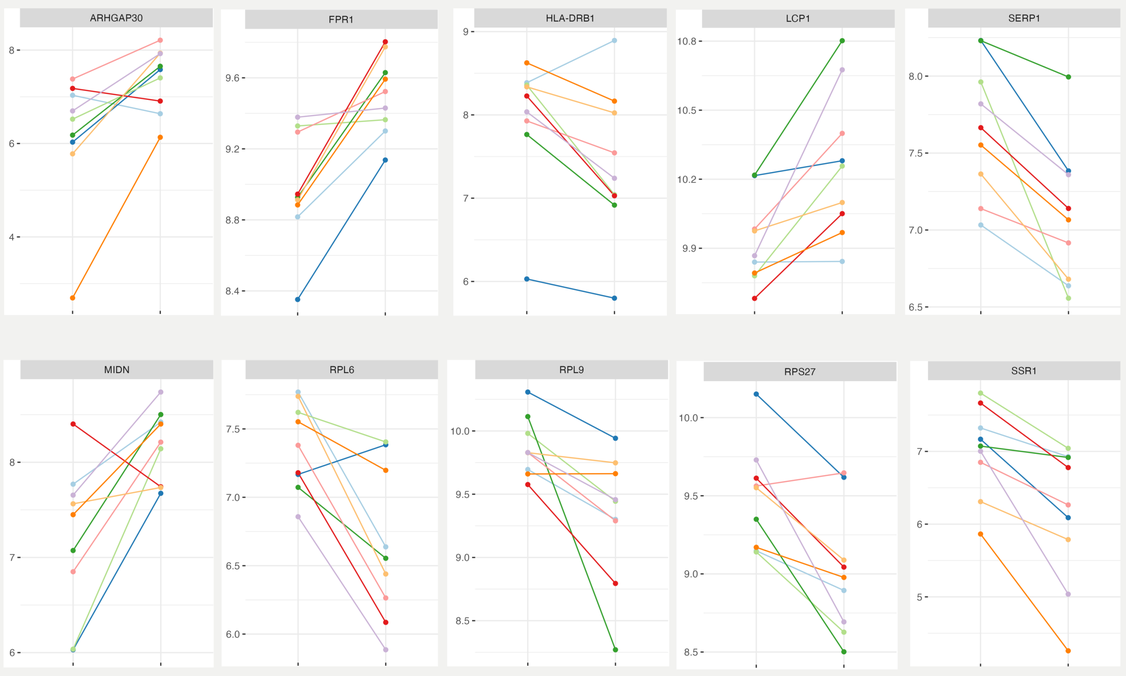


Principal Component Analysis (PCA) on gene expression data. (A) Before donor correction, clustering of samples based on the donor is observed. **(B)** After donor correction, samples collected in the morning right after waking up, and samples collected in the evening, show the greatest variability.

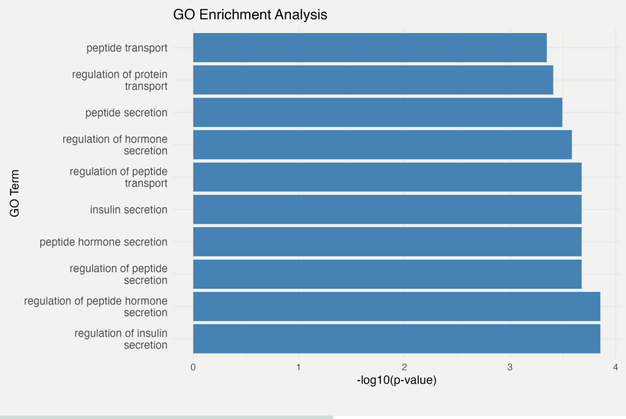


Fraction of HBA2 reads among 12 donors. Globin transcripts are the most abundant RNA molecules in blood, but in a donor-dependent manner.

Identification of common and personal changes of gene expression



Gene expression changes before and after breakfast. 10 genes were identified as significantly differentially expressed between the timepoints 'before' and '30 minutes after breakfast'. Note that some donors display a different behaviour for certain genes (see, for instance, *ARHGAP30*). Additionally, baseline levels and fold changes are donor-specific, highlighting the relevance of personalized metrics.



Top 10 significant GO terms. The GO enrichment analysis of the differentially expressed genes reveals *regulation of insulin secretion* as the most significant GO term.

Discussion & Next Steps

- **Extra efforts to deplete more globin reads are needed.** For some donors, more than 35% of the reads correspond to *HBA2*. Further optimization of our globin blockers is required to capture more meaningful transcripts.
- **Different donors display different gene expression changes, highlighting the need for personalized interpretations.** Longitudinal sampling allows to compare values from the same individual and detect in a precise and personalized way changes in their health.
- **Potential impact for cancer patients:** This technology could significantly benefit cancer patients by enabling **remote, personalized follow-ups,** reducing the need for visits to the clinic while ensuring continuous monitoring.

References

Shen X et al. (2024) Multi-omics microsampling for the profiling of lifestyle-associated changes in health. *Nat. Biomed. Eng* 8, 11–29

Subramanian A et al. (2005) Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. *PNAS* 102 (43) 15545-15550

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