

48P - Early cancer detection from liquid biopsy using cell-free RNA

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Introduction

Liquid biopsies have become increasingly important diagnostic tools for early cancer detection due to their high sensitivity and minimal invasiveness. Within liquid biopsy samples, **cell-free RNA (cfRNA)** is a promising biomarker source for early cancer detection. However, as an emerging field rife with technical challenges, cfRNA profiling for diagnostic purposes lacks gold standards for both laboratory and bioinformatic protocols.

At Flomics Biotech, we have overcome these challenges by developing a cfRNA-Seq platform that profiles human plasma cfRNA in a robust and reproducible manner. We are currently applying this platform in an ongoing pre-clinical trial (LiquiDx) with the goal of developing a **cfRNA-based multicancer early detection test**.

Methods - Flomics cell-free RNA-Seq platform

In this study, we apply our cfRNA-Seq platform (Figure 1 - Left) to plasma samples from a cohort of 1,269 donors (Figure 1 - Right). Total RNA is isolated from plasma and treated with DNase before being used as input for cDNA library preparation. cDNA libraries are sequenced in-house to obtain an average of 30 million reads per library. We analyze the cfRNA-Seq data using a curated bioinformatics pipeline with machine learning methods to extract meaningful clinical information, including cancer type-specific biomarker signatures. Stringent quality control measures are applied at multiple points during the workflow to ensure the generation and analysis of high quality sequence data.

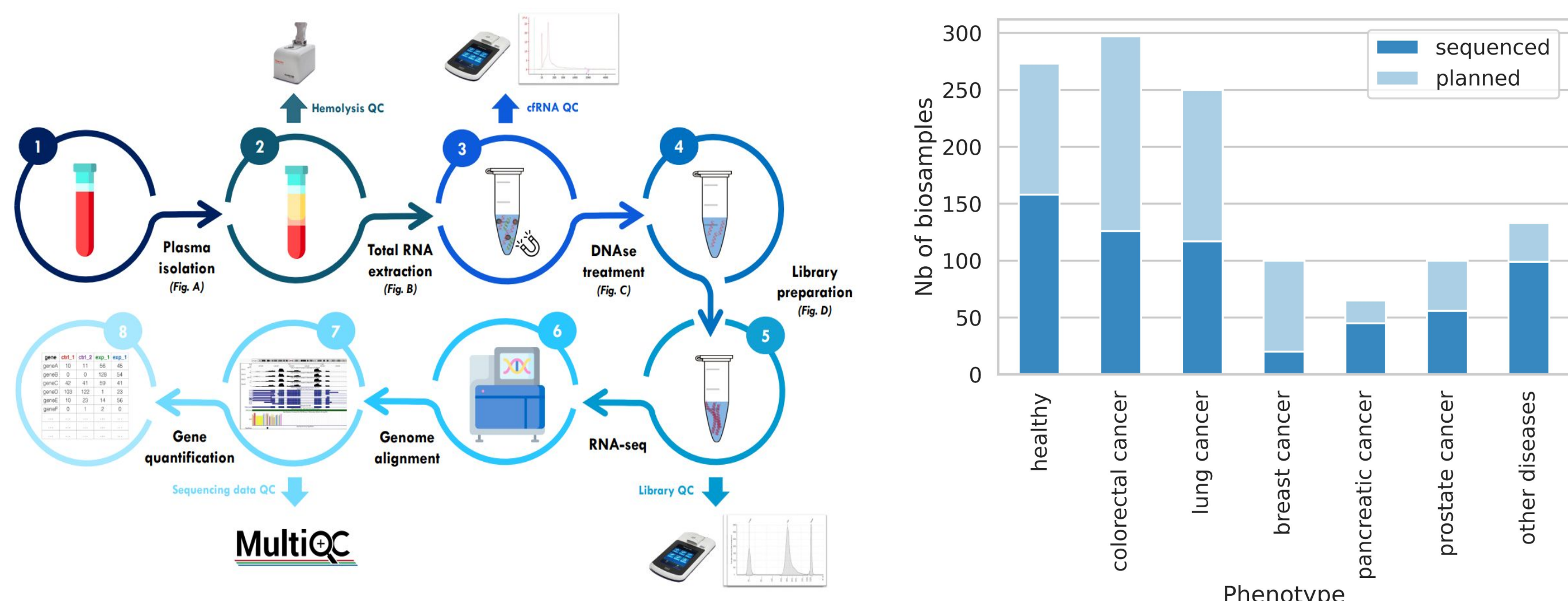


Figure 1. Left: Summary of the Flomics cfRNA pipeline from blood collection to bioinformatic analysis. QC = quality control. **Right:** Summary of the distribution of cohort biosamples across different phenotypes and biosample processing status. "Sequenced": Biosample was processed to cDNA library and sequenced. "Planned": Biosample is awaiting processing. Biosamples were obtained from both early- (stages I & II, 41%) and late-stage (III & IV, 59%) cancer patients. "Other diseases": samples from patients with diseases other than cancer of the same organs as the five cancer types.

High-quality cfRNA data for cancer biomarker discovery

We performed a comparative analysis of cfRNA data quality in published studies and data generated by Flomics (Figure 2). Due to multiple technical challenges, such as the highly fragmented nature of cfRNA and the low amount of cfRNA in biofluids, and the emerging nature of the field, cfRNA data quality varies greatly between studies. After several rounds of optimization, the Flomics cfRNA-Seq platform generates **top-level quality data** comparable to the highest quality published studies.

The high quality nature of the Flomics cfRNA-Seq platform results in a rich cfRNA transcriptomic landscape, offering a wealthy source of potential biomarkers, with more than **6000 genes detected** in 80% of the samples (Figure 3 - top left). The majority of known **oncogenes and tumour suppressor genes are detected in cfRNA** (Figure 3 - top right and bottom), showing the potential of the Flomics cfRNA-Seq platform for the detection of cancer-related signals.

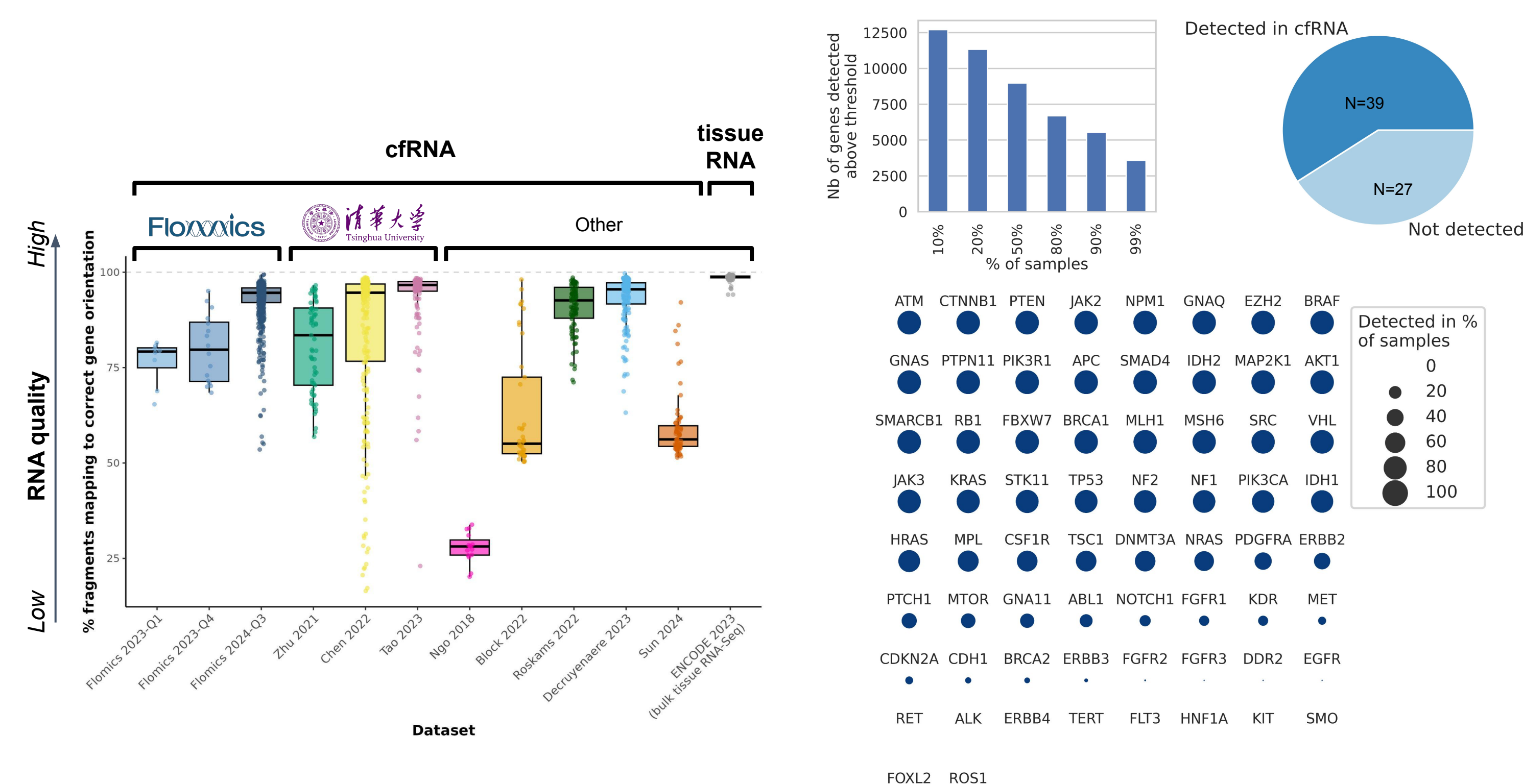


Figure 2. RNA quality of Flomics/LiquiDx libraries (left, blue) and other published datasets (right), as measured by the fraction of sequenced reads mapping to the correct annotated gene strand. Each dot corresponds to a sequenced library. Flomics cfRNA library quality compares favorably with that of gold-standard datasets, such as ENCODE tissue RNA-Seq and Tsinghua University. See the References section for further information on the published datasets.

Conclusions

- Flomics has developed a robust cfRNA platform for the monitoring of RNA molecules in biofluids.
- The Flomics cfRNA-Seq platform shows great potential for the identification of biomarkers for early cancer detection. This technology can also be applied to areas such as tracking response to therapies and infectious disease detection.
- With a diverse range of potential applications the Flomics cfRNA-Seq pipeline can have a significant impact on global health.

Acknowledgements

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Cell-free RNAs as cancer biomarkers

Whole-transcriptome profiling in plasma reveals important **differences between cancer patients and healthy individuals** (controls) (Figure 4A). We leverage these differences to (1) discover individual **cancer gene biomarkers** (Figures 4B and 4C) (2) build a **machine learning (ML) model** based on gene abundance in plasma to detect cancer (Figure 4D). The training of this model is ongoing as we generate more data (Figure 1).

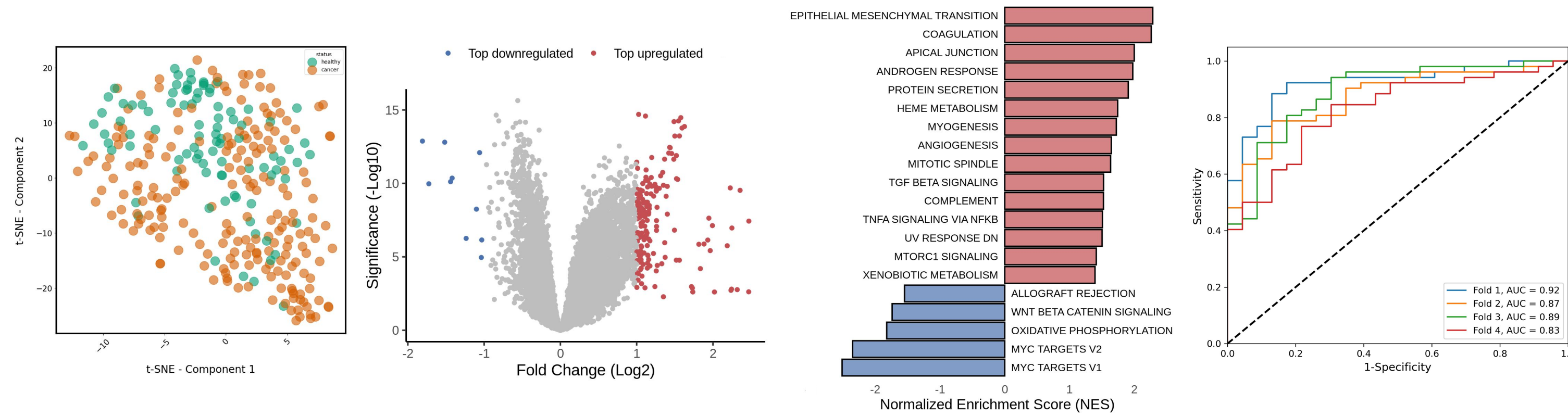


Figure 4A. Cancer and healthy donors show distinct plasma cfRNA profiles. Gene abundance-based t-SNE representation of sequenced LiquiDx samples. Samples close to each other in tSNE space have similar gene abundance profiles.

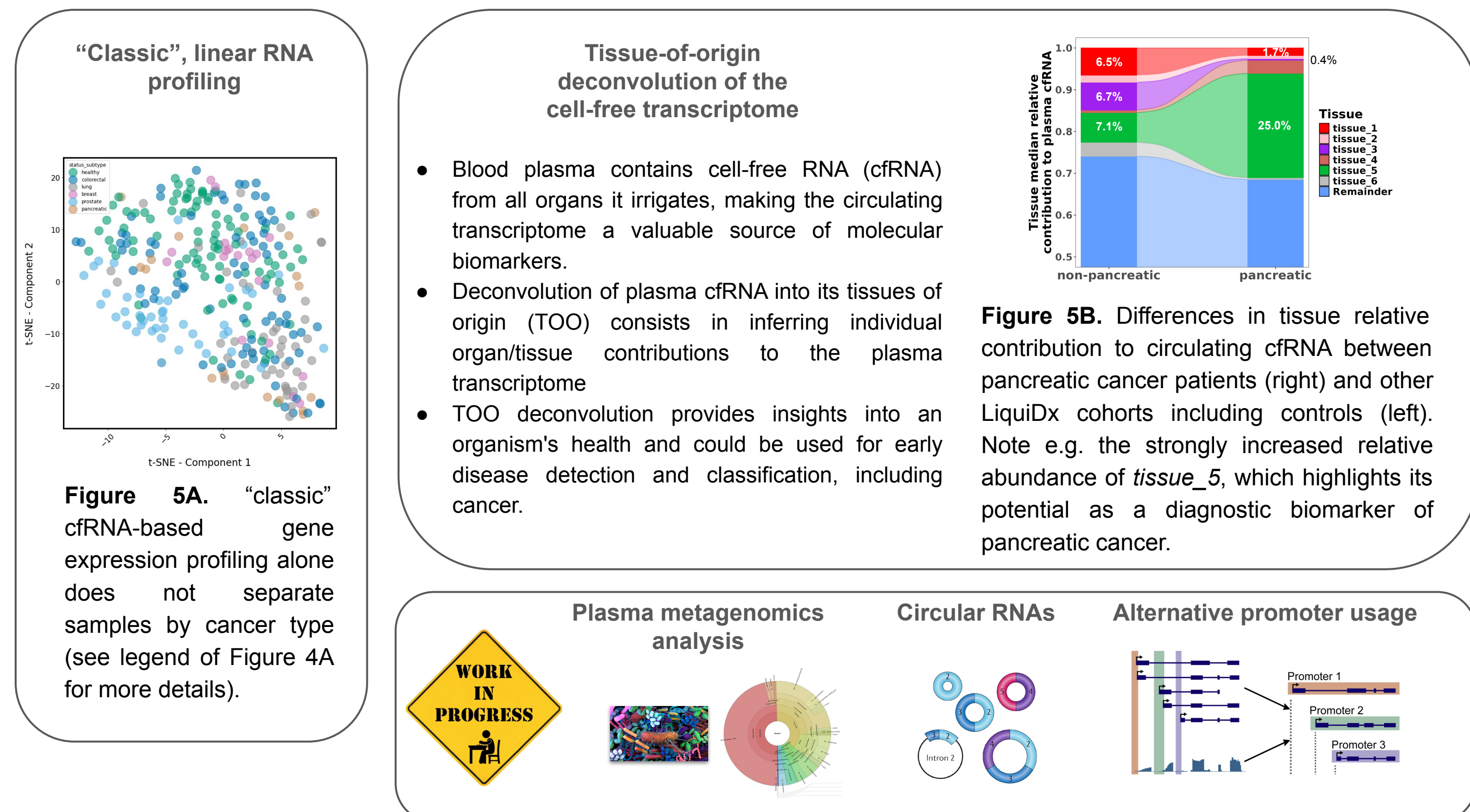
Figure 4B. Differential gene abundance analysis in LiquiDx cfRNA samples. Each dot represents a gene. Red dots represent genes that are significantly more abundant in the plasma cfRNA of cancer patients. **These genes constitute potential cancer biomarkers.**

Figure 4C. Gene Set Enrichment Analysis (GSEA) plot showing enrichment of potential cancer biomarkers. A positive NES reflects enrichment of the gene set at the top of the ranked list. Genes involved in the epithelial/mesenchymal transition, a hallmark of cancer metastasis are significantly enriched.

Figure 4D. Performance of our preliminary cancer vs healthy ML classifier, using the Gradient Boosting method. ROC curves (Sensitivity v.s. 1-Specificity) and Areas Under the Curve (AUCs) for the 4-folds cross-validation.

Advanced cfRNA-based signatures for cancer type prediction

Fine-grained separation of patients by cancer type based on gene abundance alone is highly challenging (Figure 5A). To address this, we are developing more advanced bioinformatics methods to detect cfRNA cancer type-specific molecular changes that will be combined into a final ensemble model classifier for the best accuracy in cancer type prediction.



Other cfRNA-Seq platform applications

The Flomics cfRNA-seq platform has many other applications in the context of cancer aside from early detection (Figure 6 - left). For example, it can be used to guide cancer **treatment selection** and also assess **response to cancer therapies** such as immunotherapy. **Beyond cancer**, the platform can be applied to many other diseases and research areas such as the detection of infectious diseases. During the processing of the samples described above, we detected several samples with **viral infections** including two samples with Hepacivirus C (Figure 6 - right), the ssRNA virus that causes Hepatitis C.

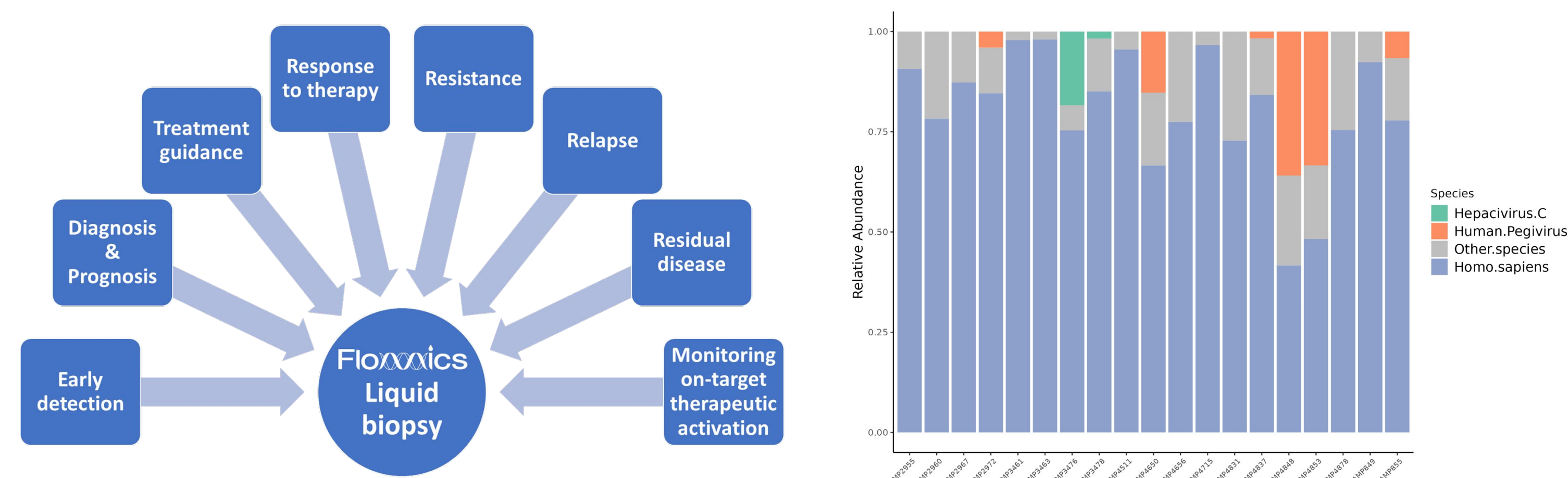


Figure 6. Left: Applications of Flomics' cfRNA-based liquid biopsy technology in the context of cancer. **Right:** Detection of viruses in human plasma samples processed using the Flomics cfRNA platform. X-axis: Samples with detectable virus infection and randomly selected plasma samples without virus infection for comparison. Y-axis: Relative abundance of reads in each sample mapping to Homo sapiens (blue), Hepacivirus C (green), Human Pegivirus 1 (orange), and all other non-human species combined (grey).

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