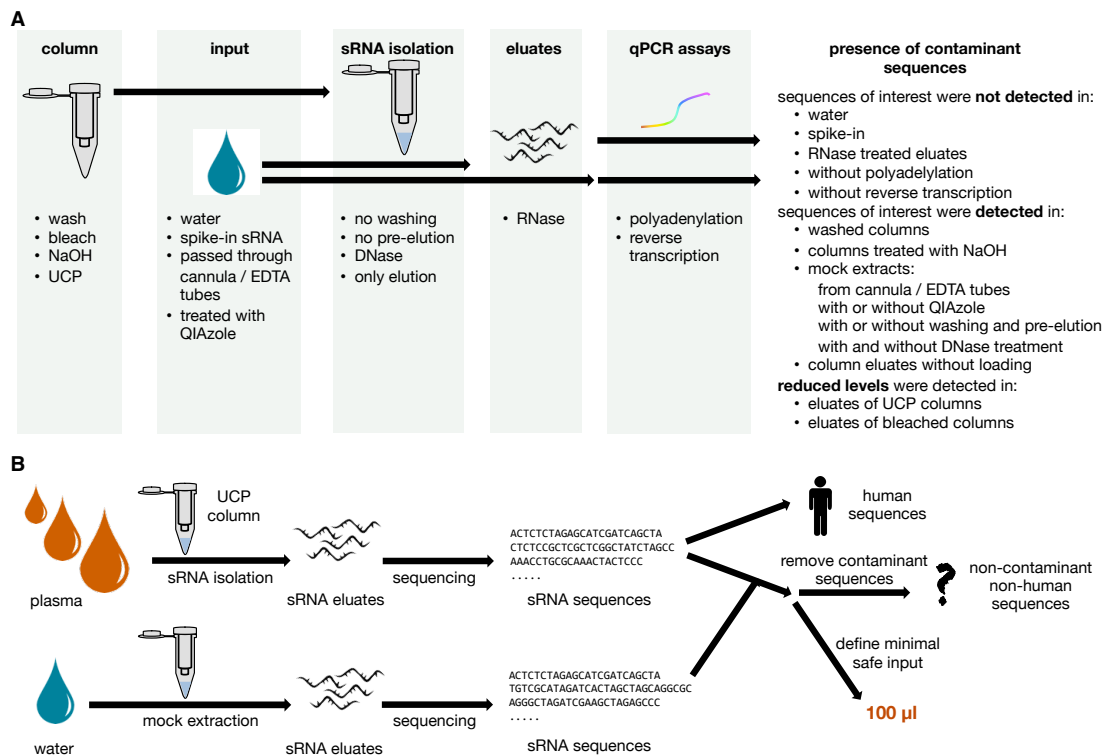
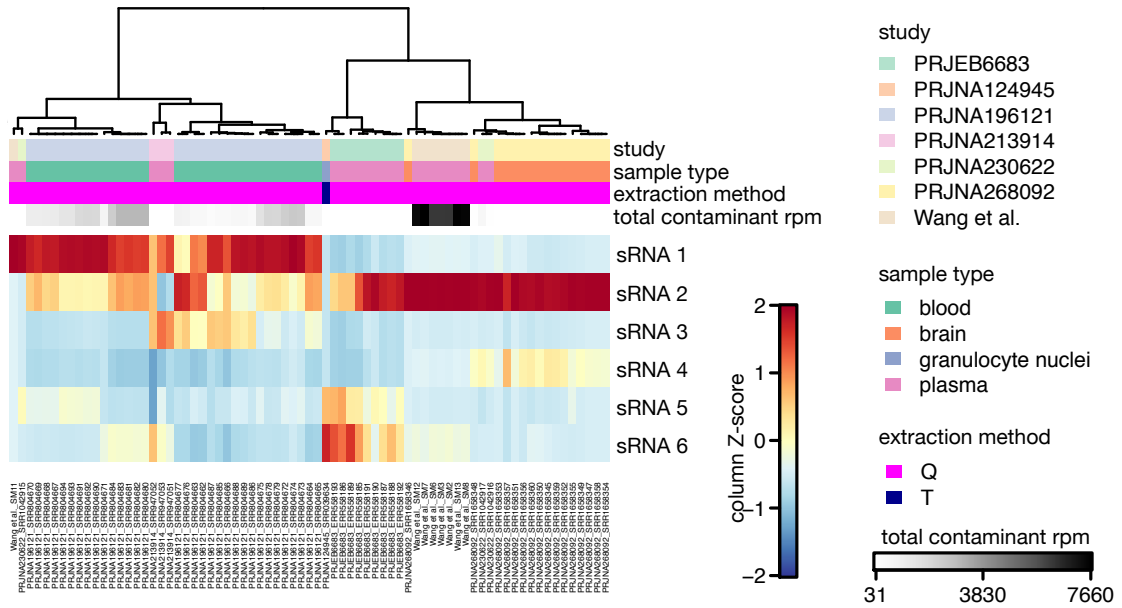


Isolation of nucleic acids from low biomass samples: detection and removal of sRNA contaminants

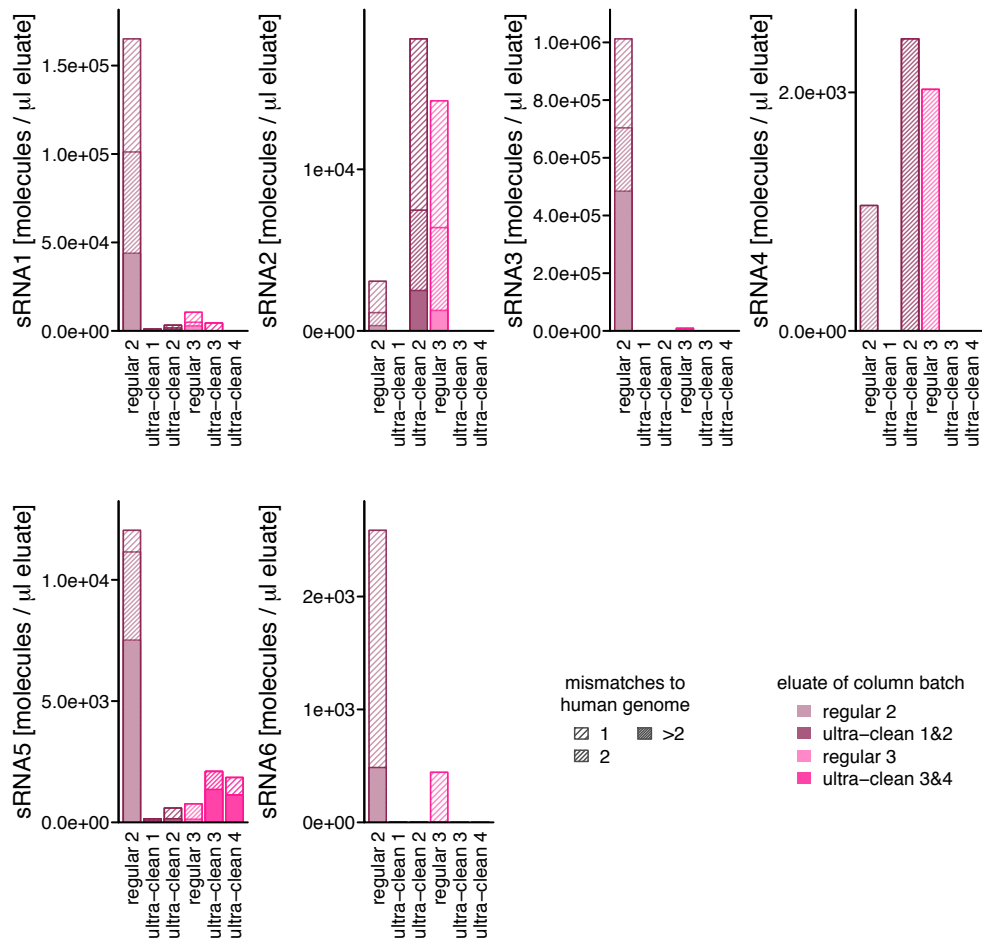
Anna Heintz-Buschart, Dilmurat Yusuf, Anne Kaysen, Alton Etheridge, Joëlle V. Fritz, Patrick May, Carine de Beaufort, Bimal B. Upadhyaya, Anubrata Ghosal, David J. Galas, and Paul Wilmes



Supplementary Figure 1. Scheme summarizing the different control experiments, the titration experiments and their outcomes. A) Tracing non-human sRNA sequences to contaminants on spin columns by variation of different steps in the isolation protocol and analysis by qPCR assays. Modifications to the steps named at the top are listed below the workflow and the outcomes are summarized at the right hand side. B) Workflow of the titration experiment to determine a minimal safe input volume for all contaminant sequences. UCP column – ultra-clean column.



Supplementary Figure 2. Detection of contaminants in published datasets. Heatmap showing the relative abundances of the confirmed contaminant sequences in published sRNA sequencing data of low-biomass samples. Only samples for which any of the confirmed contaminants were detected are shown. Extraction methods: Q: regular QIAGEN miRNeasy; T: TRIZOL. rpm: reads per million.



Supplementary Figure 3. Detection of contaminants in eluates of regular and ultra-clean RNeasy columns. Two batches of regular miRNeasy columns and four batches of ultra-clean RNeasy columns were compared. Results are based on sRNA sequencing data of mock-extracts, normalized to the detected levels of spike-in synthetic RNAs. The different shadings represent reads mapping to the human genome with 2, 1, or 0 mismatches and the different column batches are coloured in the same colours as in main figure 3, as indicated in the legends.

Additional supplementary data:

Supplementary Table 1: list of the generated datasets and analysed published datasets.

Supplementary Table 2: list of the species whose reference genomes and cDNA collections were used in the initial analysis.

Supplementary Table 3: potential exogenous sRNA sequences detected in human plasma after removal of contaminants.