Characterization of RNA from extracellular vesicles

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Introduction

Extracellular vesicles (EVs or "vesicles") are a rich source of high-quality RNA, released by all virtually all cells and present in all biofluids. Many current protocols to isolate vesicles use ultracentrifugation; however, development of faster, more convenient methods with high specificity for vesicles and good RNA quality is an area of active research. Here we present a characterization of vesicle RNA isolated from plasma and serum by several different methods, including ultracentrifugation and a novel spin-column-based method. The RNA was subjected to electrophoresis, RT-qPCR arrays and NextGen Sequencing to demonstrate the utility of vesicle RNA as a source for blood-based biomarkers.

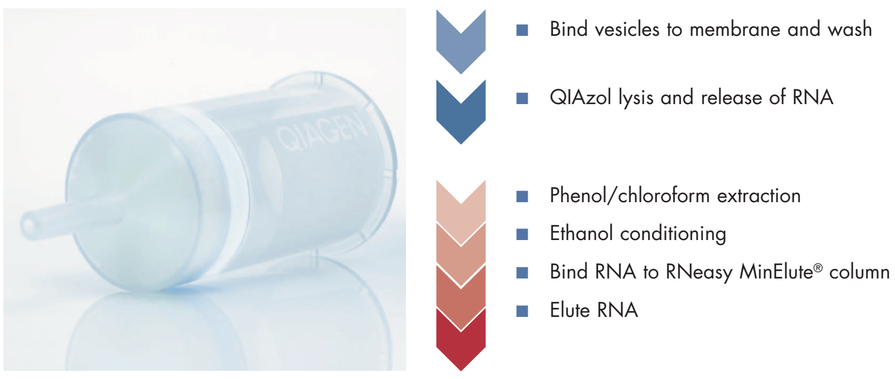

Figure 1. The new exoRNeasy Serum/Plasma Maxi Kit. Disposable spin column to purify vesicles and workflow of the exoRNeasy Serum/Plasma Maxi Kit.

Small and large RNAs in extracellular vesicles

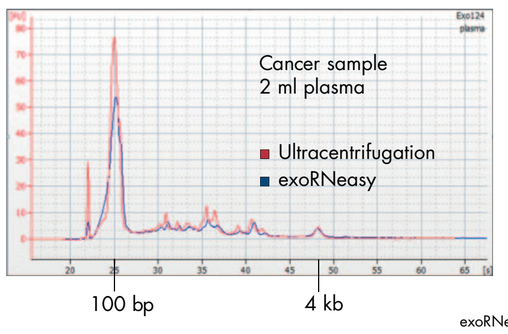

Figure 2. exoRNeasy and ultracentrifugation give similar RNA yields from vesicles present in blood plasma. Bioanalyzer sizing of vesicle-derived RNA purified by two methods. The plasma was pre-filtered (0.8 µm) to exclude larger particles and subjected to either ultracentrifugation, the current gold standard of vesicle isolation, or the exoRNeasy procedure. Both methods purify RNA of similar size and yield.

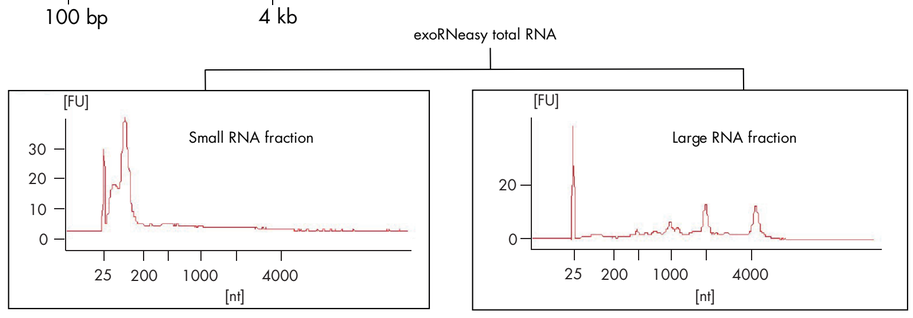

Figure 3. EVs contain both, small and large RNAs. RNA from 2 ml of pre-filtered plasma was separated into a large and small fraction using different ethanol concentrations for binding to the RNeasy purification column. Subsequently, both fractions where analyzed using a Bioanalyzer Pico assay. The presence of sharp ribosomal RNA peaks demonstrates the purification of large, intact, non-degraded RNAs from EVs.

Integrity of mRNA in vesicles and detection of oncogenes in different sample volumes

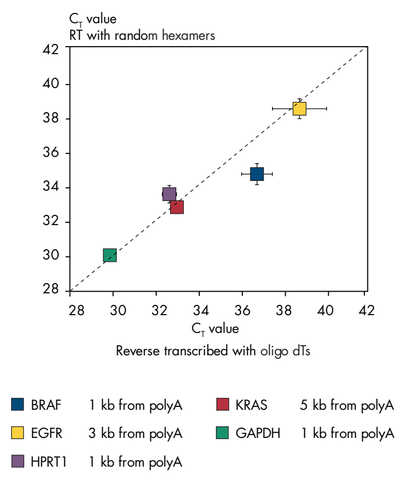

Figure 4. Extracellular vesicles contain full-length mRNAs with intact poly A tails. RNA from 2 ml of pre-filtered plasma was extracted with exoRNeasy and subjected to reverse transcription with the superscript III enzyme using either random hexamers or oligo-dTs and 6 replicate reactions for each. Both methods yield comparable C_Ts in RT-qPCR, demonstrating that the quantified mRNA transcripts are not degraded.

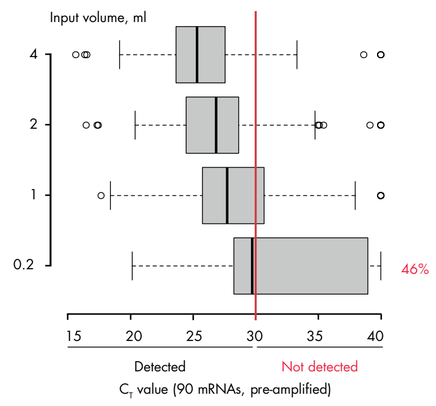

Figure 5. mRNAs from known oncogenes are readily detected in high volumes of plasma. RNA from various volumes of plasma were isolated with exoRNeasy, reverse transcribed and pre-amplified using the RT² PCR system and detected using the Human Cancer Pathway Finder PCR Array. At 0.2 ml only 46% of the mRNAs are robustly detected (C_T<30) but the isolation with exoRNeasy can be scaled up to 4 ml with linear increase in C_T signal and mRNA detection rate.

Separation of vesicular from non-vesicular RNA

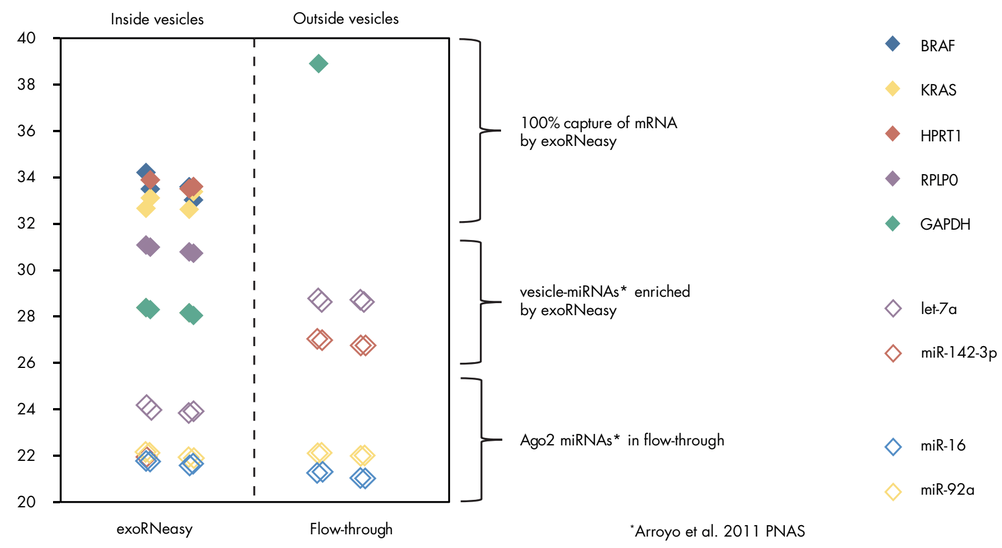

Figure 6. exoRNeasy captures all mRNA and vesicle-specific miRNAs in plasma. RNA from 0.2 ml of pre-filtered plasma was isolated with exoRNeasy and the flow through of the exoEasy column was used in direct lysis. Shown are raw C_T values from RT-qPCRs with rows as replicate isolations and colored diamonds as replicate qPCRs.

RNA recovery with different methods and detection of oncogenic mutations

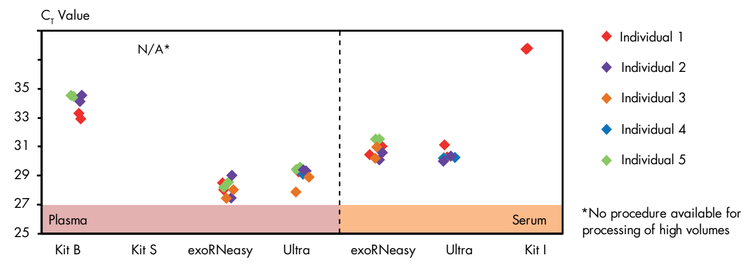

Figure 7. exoRNeasy enables extraction from high volumes of plasma and serum. Vesicles from 4 ml of plasma or serum were isolated with exoRNeasy, ultracentrifugation (Ultra) and three commercially available methods based on filtration (Kit B) or precipitation (Kit S, Kit I). Only exoRNeasy and ultracentrifugation efficiently recover RNA from high sample volumes.

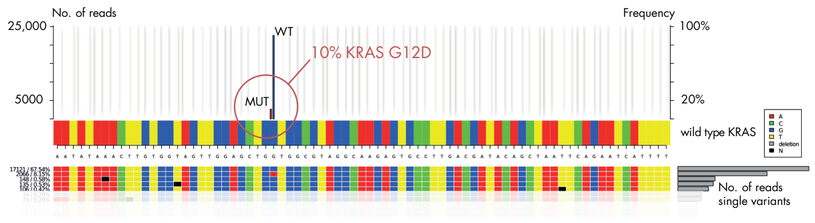

Figure 8. NextGen Sequencing detects somatic mutations from CRC in vesicle RNA. A 2 ml sample of pre-filtered plasma was drawn from a patient with KRAS G12D positive colorectal cancer (CRC). RNA from vesicles was isolated using exoRNeasy and analyzed with targeted re-sequencing on an Illumina® MiSeq®. Over 10% of all reads that matched to the KRAS gene carry the c.35 G>A/p.G12D mutation previously identified in the primary tumor.

Conclusions

- The exoRNeasy Serum/Plasma Maxi Kit can be used to extract high-quality RNA from plasma EVs using a fast and convenient spin-column procedure.
- EVs contain small and large non-degraded mRNAs.
- EVs contain plasma mRNA and a specific fraction of miRNA.
- EVs enable detection of somatic mutations and oncogene expression.
- Sensitivity of oncogenic transcript detection is highly dependent on sufficient sample volume

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The applications presented here are for molecular biology applications. They are not intended for the diagnosis, prevention or treatment of a disease.

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