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Comparison of Different Methods for miRNA Purification from Plasma

Gabriele Christoffel[1], Heike Glowatz[1], Yasmin Fries[1], Verena Schramm[1], Francesca Di Pasquale[1], Jonathan M. Shaffer[2], Markus Sprenger-Haussels[1]

[1] QIAGEN GmbH, Hilden, Germany; [2] QIAGEN Sciences Inc., Frederick, MA, United States

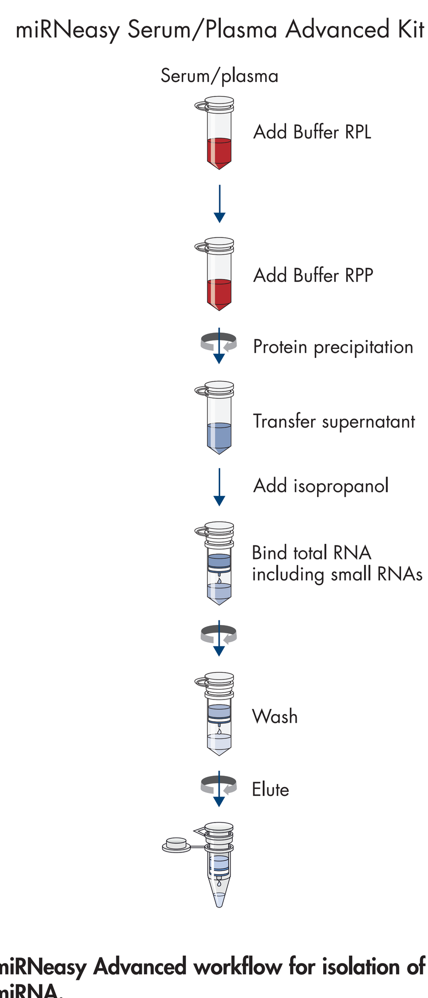
Introduction

MicroRNA profiling in serum and plasma samples holds high expectations as a noninvasive way of discovering potential biomarkers for a broad spectrum of diseases and biological processes. However, obtaining true microRNA profiles from such samples can be challenging, due to high concentrations of protein, including nucleases and other components, which not only interfere with the isolation of miRNA, but also with downstream analysis.

Here, we present complete workflows from sample preparation to analysis and biomarker detection by next generation sequencing (NGS), as well as qPCR.

To analyze the risk of external contamination introduced by the purification method and to determine the minimal sample input volume of plasma, miRNA was purified from different volumes of plasma. miRNAs with higher read counts in samples with lower input volume (compared with samples with higher input volume) were rated as potential contaminations.

In this study, we compare different sample preparation methods for sensitivity and consistency of the recovered miRNA.



miRNeasy Advanced workflow for isolation of miRNA.

Materials and Methods

Blood from healthy donors was collected in EDTA blood collection tubes. After separation of plasma and removal of residual cells and debris, miRNA was purified using different methods. Relative abundance of selected miRNAs was compared by qPCR using the miScript® System.

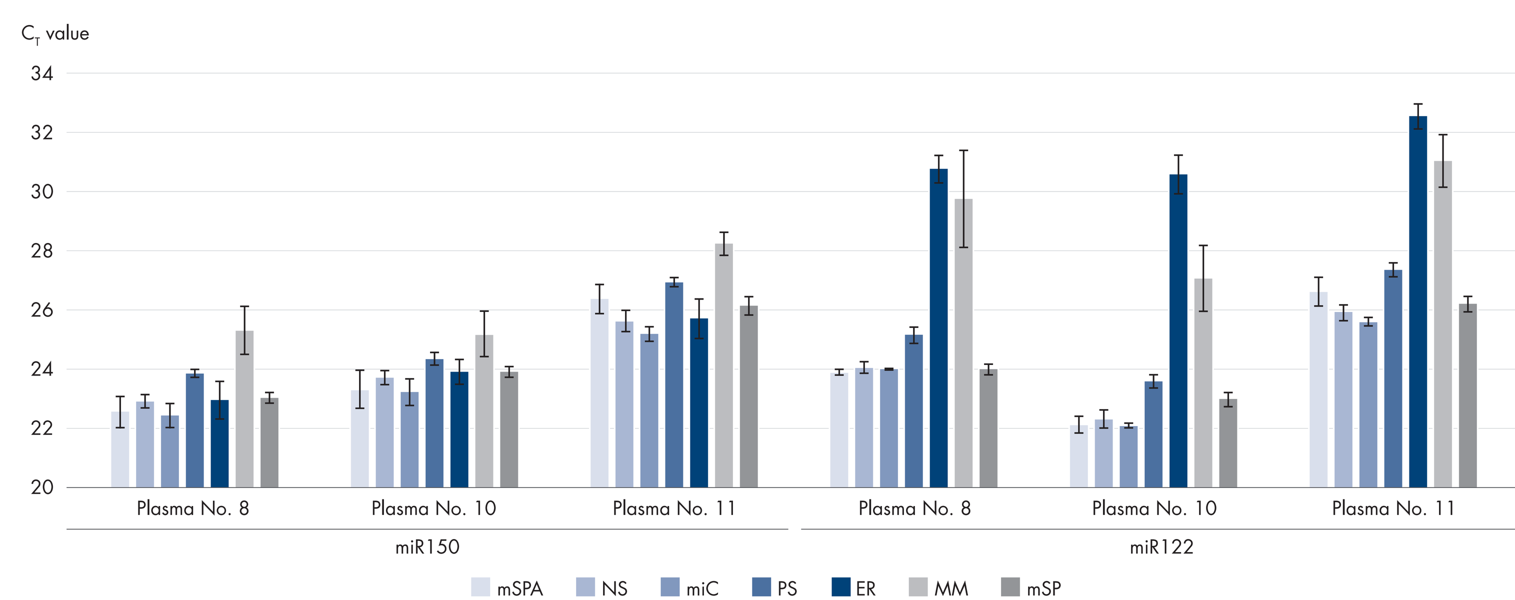
In addition, we analyzed the recovered miRNA by NGS using unique molecular indices (UMI; QIAseq™ miRNA Library Kit). miRNAs detected at higher frequencies in eluates purified from 25 µl plasma, rather than from 200 µl plasma, were considered as potential contaminations introduced by the purification method and/or the library preparation.

Sample preparation kits investigated, input and elution volumes.

Kit	Abbreviation	Company	Plasma (µl)	Elution volume (µl)
miRNeasy Serum/Plasma Advanced Kit	mSPA	QIAGEN	200	20
NucleoSpin® miRNA Plasma Kit	NS	Macherey Nagel	300	30
miRCURY™ RNA Isolation Kit	miC	Exiqon (QIAGEN)	200	50
Plasma/Serum RNA Purification Mini Kit	PS	Norgen	200	20
exoRNeasy Serum/Plasma Midi Kit	ER	QIAGEN	1000	20
MagMax™ mirVana Total RNA Isolation Kit	MM	Thermo Fisher Scientific	100	50
miRNeasy Serum/Plasma Kit	mSP	QIAGEN	200	50

miRNA Recovery qPCR

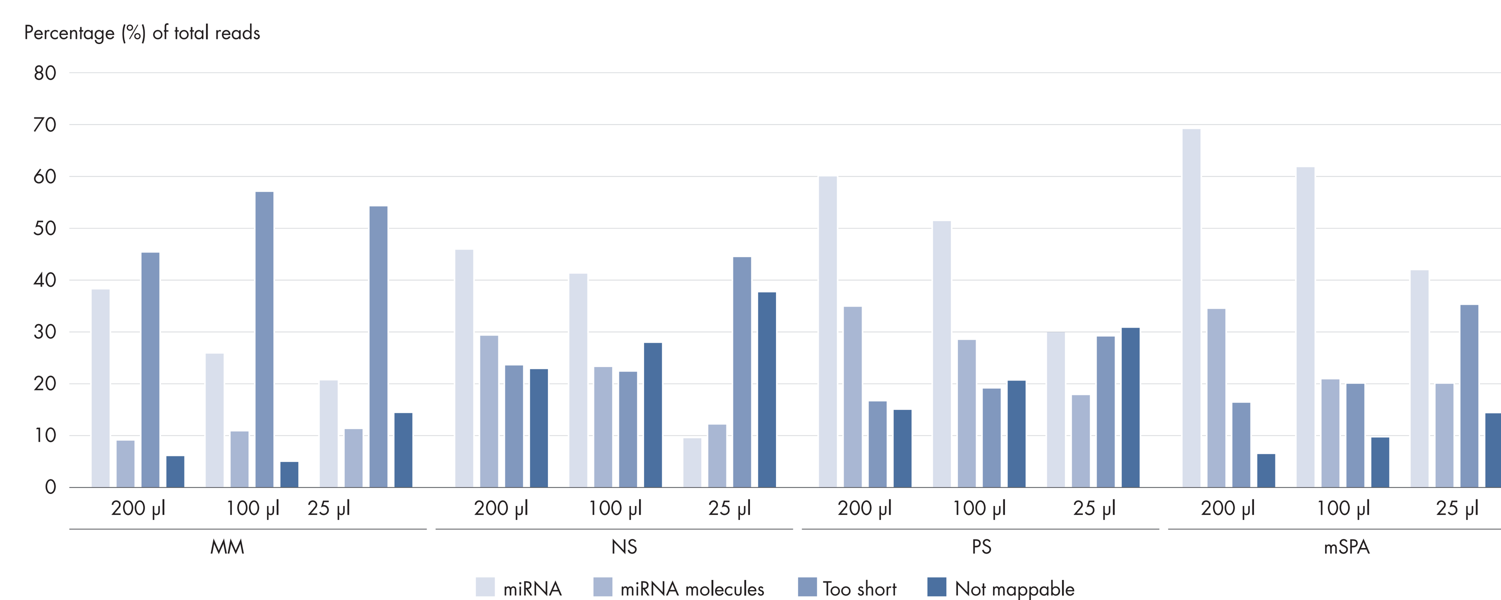
miRNA was purified from blood from three different healthy donors using various purification methods (see table on previous panel). Depending on the elution volume, an equal amount of the recovered RNA was applied in a total volume of 25 µl for cDNA synthesis using the miScript system, followed by a PCR detecting the miR122a and miR150 miRNA, respectively. The miRNeasy Serum/Plasma Advanced (mSPA), NS and miC methods generally showed the best recovery for both tested miRNAs (i.e., lowest C_T value). As expected, RNA isolated using the exoRNeasy method shows equal performance detecting miR150 (inside of extracellular microvesicle; EV) and higher C_T value when targeting miR122 (outside of EV).



miRNA recovery qPCR results.

miRNA Next Generation Sequencing

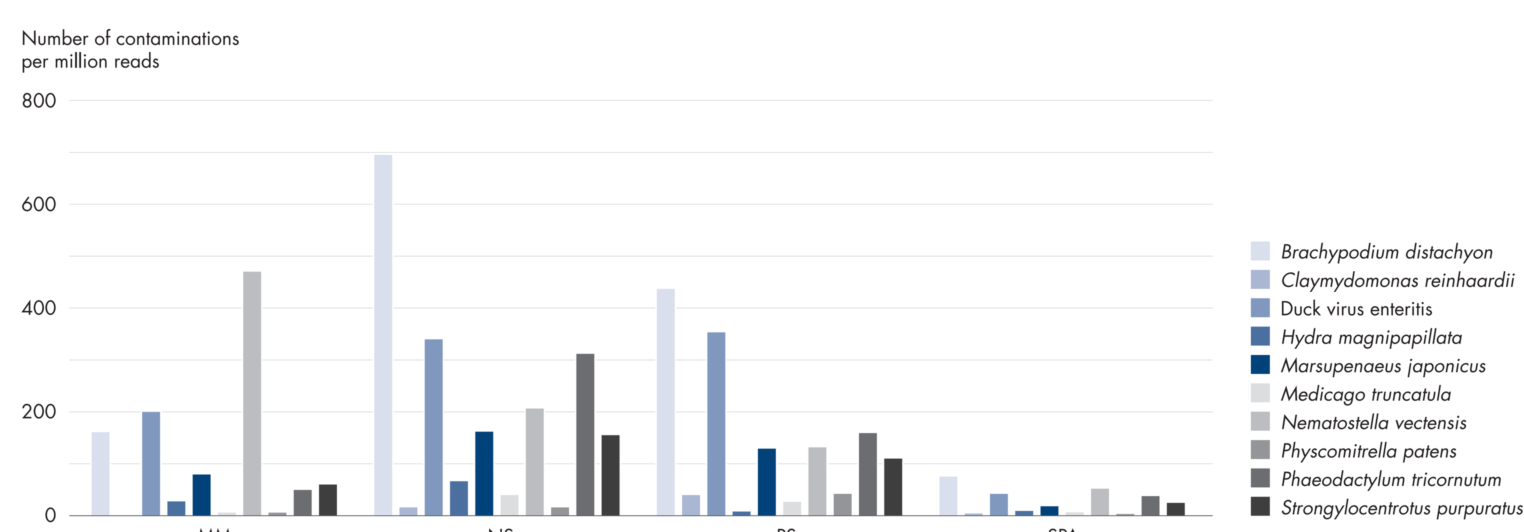
Different purification methods were compared using different input volumes of plasma (25–200 µl). Overall, the miRNeasy Serum/Plasma Advanced method showed the lowest number of "unmappable" reads and "too short reads", together with the highest number of "miRNA" reads.



NGS run analysis showing miRNA total reads, miRNA reads UMI-collapsed, "too short" reads and "unmappable" reads.

Contamination Issues

miRNA was purified from varying plasma volumes using several methods. Contaminations were identified by comparing miRNA frequencies of different input volumes. If sequences originate from contamination, their frequencies will be higher in samples with lower plasma volumes. The figure shows the UMI-collapsed number of potential contaminations per million reads using different isolation kits. Samples prepared with the miRNeasy Serum/Plasma Advanced method show the lowest amount of potential contamination.



NGS run analysis showing miRNA total reads, miRNA reads UMI-collapsed, "too short" reads and "unmappable" reads.

Conclusions

We compared different miRNA purification methods for isolation efficiency and compatibility with different assay formats (real-time PCR and NGS). In addition, we examined potential contamination introduced by the purification method and/or the NGS downstream respectively.

In conclusion:

- Real-time PCR showed that the miRNeasy Serum/Plasma Advanced, NS and miC methods performed best.
- As expected, the exoRNeasy method shows equal performance detecting miRNA inside EVs and lower performance outside of EVs.
- NGS shows more diverse results, but the miRNeasy Serum/Plasma Advanced method gave the most UMI and total miRNA reads and least "too short" or "unmappable" reads, followed by NS and PS methods.
- NS and PS methods, however, showed a higher contamination rate compared with the miRNeasy Serum/Plasma Advanced method.

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