Isolation of small RNA species from PAXgene Blood RNA Tubes

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Introduction

Sampling of blood is a standard procedure in most clinical trials and many diagnostic testing procedures. Pathological conditions in organs and remote tissues are often detectable in gene expression profiles from blood samples. Artificial modifications of the RNA content and profile of a given blood sample between collection and isolation procedure caused by degradation and gene induction are well documented (Rainen et al. 2002 Clin Chem 48: 1883-90; Müller et al. 2002 Leukemia 16: 2395- 99). Doubtful results are the consequence especially for very sensitive analysis methods like qRT-PCR assays and gene chip experiments. Thus the need for stabilisation of cellular RNA species to freeze the gene expression profile at the time of blood collection is widely accepted and the PAXgene Blood RNA stabilisation and isolation systems are commonly used to address this problem.

Since the standard PAXgene Blood RNA protocols were designed for mRNA purification the isolation of miRNA from PAXgene Blood RNA tubes is not very efficient, as the binding conditions are not optimized for small RNA species. A first attempt to optimize these conditions and to allow a more efficient purification of small RNAs from PAXgene Blood RNA tubes was published recently (Kruhøffer et al. 2007 Vol 4(4): 452-8). Due to several drawbacks of this method, we developed a new chemistry to achieve optimal yields of small RNAs from PAXgene Blood RNA Tubes. The corresponding protocols allow either a manual or an automated isolation via the QIAcube (QIAGEN, Fig. 1) of small as well as large RNA species.

Disclaimer: PAXgene Blood miRNA system is intended for Research Use Only. Not for use in diagnostic procedures.

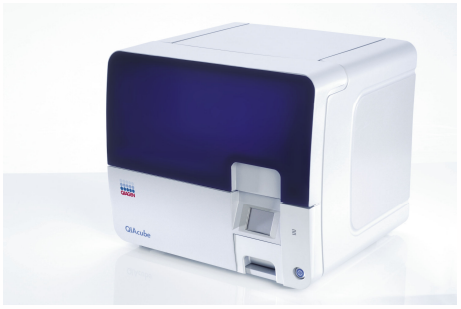

Figure 1. QIAcube instrument

Material and methods

Blood was collected in PAXgene Blood RNA Tubes (PreAnalytiX), stored for 20-24 h at RT (room temperature) and frozen at -20° C. The tubes were thawed 2h at RT before processing. Beside the new PAXgene Blood miRNA Kit (Fig. 2) we used the manual PAXgene Blood RNA Kit (PreAnalytiX) as reference. The quantity and purity of the RNA samples were analyzed by spectrophotometric analysis. The quality and enrichment of small RNAs were determined on an Agilent 2100 Bioanalyser by using the Nano and small RNA Labchip (Agilent) as well as with classical gel analysis. Purified RNA was analyzed for genomic DNA contamination and the presence of PCR inhibitors. As downstream methods different quantitative RT-PCR assays for DNA, mRNAs and especially miRNAs based on SYBR green or probes (QIAGEN or Applied Biosystems) were used. These assays were performed by using ABI7700 or 7900 PCR systems.

RNA quantity and quality

We found total RNA yields ≥ 3 µg for 99 % of all samples with a WBC of 4.8-11 x 10^6/ml. The average yield was 6-7µg per tube. The RNA purity (260nm/280nm ratio) was between 1.8-2.2 for 95 % of all samples. Very low amounts of gDNA (< 1%) were present in the eluates and they showed no interference with qRT-PCRs when detecting different mRNA transcripts (data not shown).

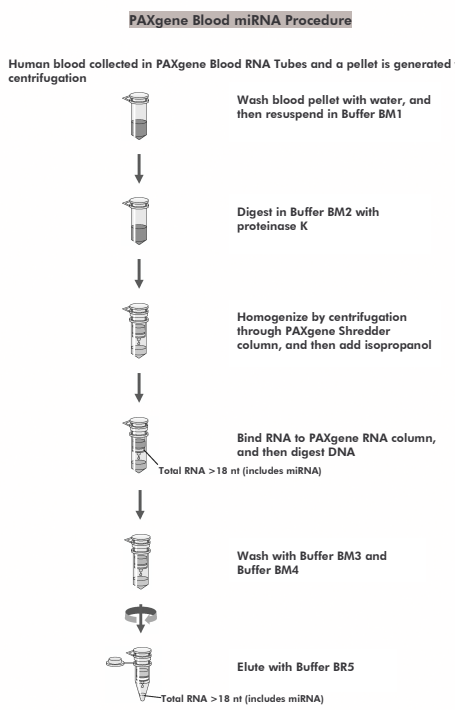

Figure 2. PAXgene Blood miRNA workflow

Results – SybrGreen based assays

- All four miRNAs were clearly enriched in samples processed with the PAXgene Blood miRNA Kit

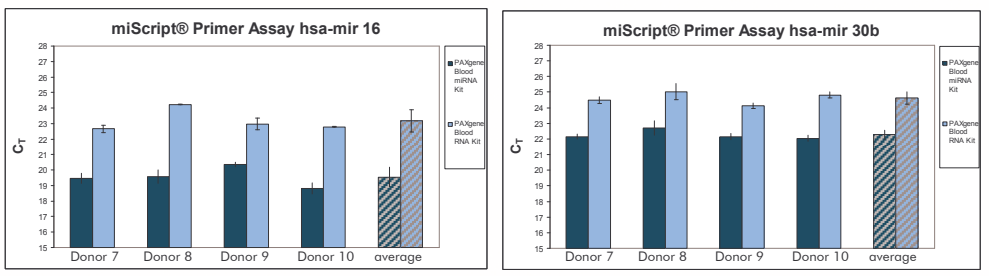

Figure 5. Results of four different miScript® Primer Assays comparimg the eluates generated with the PAXgene Blood miRNA or the PAXgene Blood RNA Kit.

Results – Probe based assays

- All four miRNAs were clearly enriched in samples processed with the PAXgene Blood miRNA Kit.

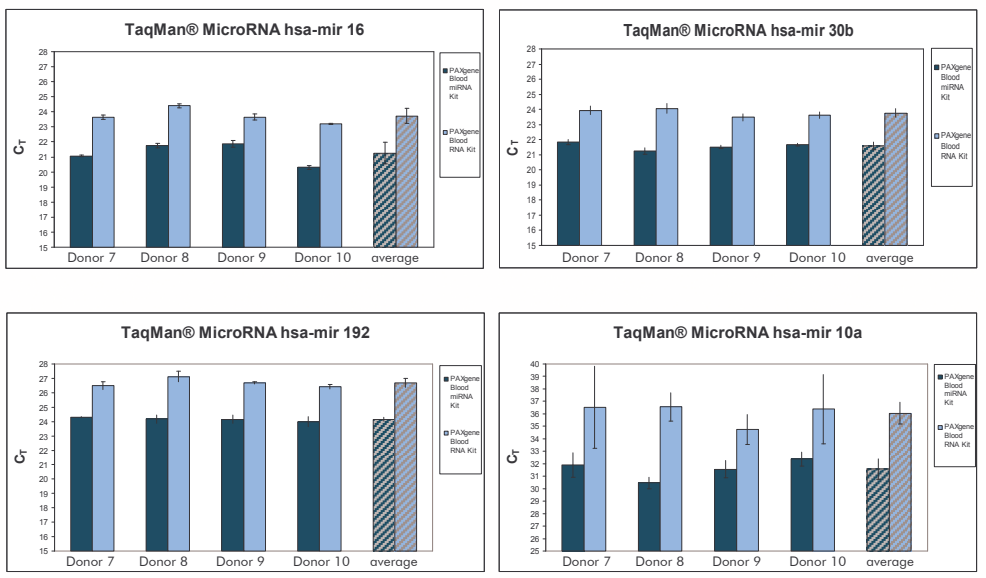

Figure 6. Results of four different TaqMan® MicroRNA assays comparimg the eluates generated with the PAXgene Blood miRNA or the PAXgene Blood RNA Kit.

Results – Enrichment of small RNAs

- Small RNAs were clearly enriched in samples processed with the PAXgene Blood miRNA Kit

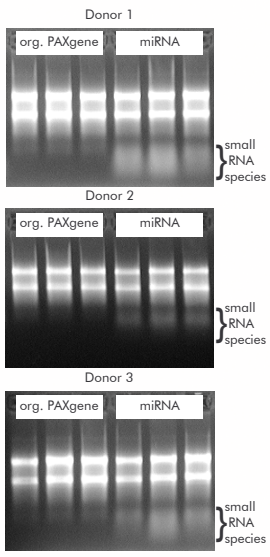

Figure-3. Analysis of eluates by agarose gel electrophoresis.

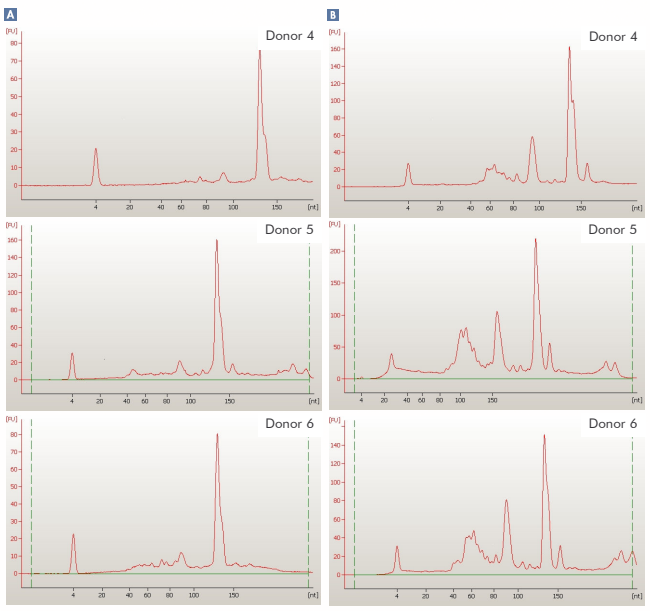

Figure 4. Eluate analysis with the Agilent Small RNA LabChip. The Y-axis represents fluorescence units (FU) and the X-axis nucleotides (nt). A PAXgene Blood RNA eluates; B PAXgene Blood miRNA eluates.

Summary and conclusions

- Purification using the PAXgene Blood miRNA Kit provided RNA with high purity and very low genomic DNA contamination.
- Clear enrichment of small RNA species in eluates generated with the PAXgene Blood miRNA Kit could be shown by electrophoretical methods.
- Clear enrichment of all tested miRNAs could be shown by using two different realtime RT-PCR systems.
- These results show that the dedicated isolation procedures for small RNAs based on the PAXgene Blood RNA Tube result in high quality enrichment of these RNA species which are ready for direct use in sensitive down-stream applications like real-time RT-PCR.