High-throughput automation for RNA isolation from blood stabilized in PAXgene™ Blood RNA Tubes

Thorsten Voss, Ralf Wyrich, Daniel Langendörfer, Thomas Rothmann, and Uwe Oelmüller

QIAGEN R&D Department, QIAGEN GmbH, Hilden, Germany

PreAnalytiX GmbH, Hombrechtikon, Switzerland

Introduction

Ex vivo changes in RNA content and profile in blood samples post-blood draw are a major issue for diagnostic applications. If RNA is isolated after cellular degradation, gene expression data from these samples can be misleading. PreAnalytiX GmbH, Hombrechtikon, Switzerland (PreAnalytiX) and QIAGEN R&D Department, QIAGEN GmbH, Hilden, Germany (QIAGEN), have designed and developed the PAXgene Blood RNA Tube. The system provides a convenient solution for collection of whole blood and immediate stabilization of the cellular RNA profile. PAXgene Blood RNA Tubes contain proprietary reagent composition based on a patented stabilization technology. This reagent combination protects RNA from degradation by RNases and minimizes ex vivo changes in gene expression. PAXgene Blood RNA Tubes are intended for the collection of human whole blood and stabilization of cellular RNA for up to 3 days at 18–25°C up to 5 days at 2–8°C. Currently available data show that PAXgene Blood RNA Tubes provide reproducible, high-quality RNA as determined by downstream applications such as real-time RT-PCR and GeneChip array analysis.

Methods and materials

Blood was collected in PAXgene Blood RNA Tubes (PAXgene MDx), stored for 20–24 h at room temperature (18–25°C) and then frozen at −20°C. The frozen samples were thawed for 2 h at room temperature before processing. For automated cell isolation, the PAXgene Blood RNA MDx Kit (PAXgene MDx Kit) was used on both the BioRobot MDx and the BioRobot Universal System (QIAGEN). As a reference, the manual PAXgene 96 Blood RNA Kit or for Affymetrix GeneChip gene expression experiments, the PAXgene Blood RNA Kit was used. The quality and quantity of the RNA samples were analyzed by spectrophotometric analysis (SpectraMax® spectrophotometer, Molecular Devices) and on an Agilent 2100 bioanalyzer. Downstream gene expression analyses were performed using quantitative, real-time RT-PCR assays and Affymetrix GeneChip Global Gene Expression Arrays.

Results

Results — RNA yields

- Average yield: 7 µg/tube
- 3300 tubes processed with final protocol in 42 runs (1740 tubes on the BioRobot MDx; 1560 tubes on the BioRobot Universal System).
- Samples with yields ≥3 µg — only 0.9% of samples with yields ≥3 µg.

Results — RNA purity

- Reproducibility: average CV 16%
- Total failure rate: no RNA visible in denaturing agarose gel electrophoresis for only 0.09% of all samples.
- Reproducibility: CV 2.25% between units and different donor samples for 99% of all samples.

Results — GeneChip array analysis

- GeneChip array analysis gives reproducible high yields of RNA from blood stabilized in PAXgene Blood RNA Tubes.
- Purification using the PAXgene Blood RNA MDx Kit provided RNA with high purity and very low genomic DNA contamination, ensuring reliable performance of these RNA samples in Affymetrix GeneChip array analyses.

Conclusions

- Purification using the PAXgene Blood RNA MDx Kit provided RNA with high purity and very low genomic DNA contamination, enabling high-throughput RNA isolation and purification for gene expression analysis.

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