

INFLUENCE OF TOTAL RNA INTEGRITY ON miRNA QUANTIFICATION

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Introduction

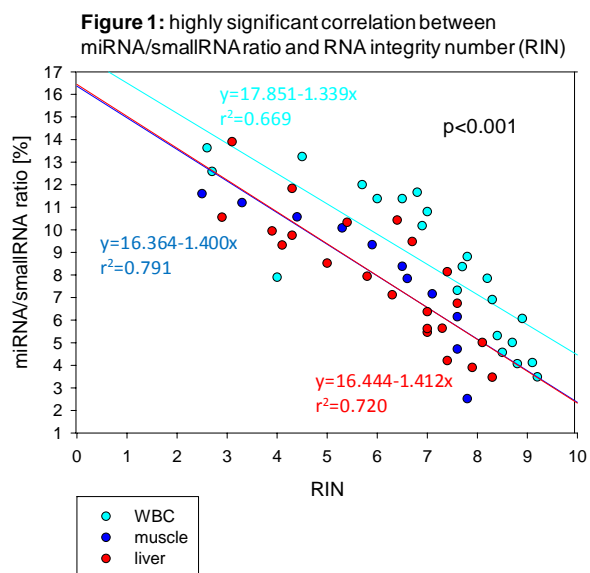
The analysis of RNA quality is a valuable tool in classical gene expression profiling via qRT-PCR and microarray analysis. This technique may also be integrated in the routine analysis of new applications like the investigation of miRNA expression. Agilent Technologies offers a new application for the 2100 Bioanalyzer making it possible to analyze small RNA with the lab-on-a-chip technology. By now this chip provides the only possibility to quantify miRNA in absolute amounts [pg] and as a percentage of small RNA [%].

Ongoing RNA degradation is accompanied by the formation of small RNA fragments and though possibly influences the miRNA quantification leading to an overestimated miRNA amount. Aim of the study was to investigate the influence of artificially caused RNA degradation on miRNA quantification and miRNA expression.

Methods

RNA and miRNA were extracted from different bovine tissues [liver, muscle, white blood cells (WBC)] in six replicates (n=6) with the miRNeasy system (Qiagen). Total RNA quantification was done using the NanoDrop (peqLab). RNA quality analysis and miRNA quantification were done with the 2100 Bioanalyzer using the RNA Nano Assay and the smallRNA assay (Agilent Technologies). Gene expression analysis for mRNA and miRNA was undertaken with real-time qRT-PCR on the realplex platform (Eppendorf). For mRNA expression analysis Ubiquitin (UBQ), Lactat-dehydrogenase (LDH) and Caspase 3 were measured. miR-1, miR-122 and miR-195 were investigated in miRNA expression analysis.

Figure 1



Results and discussion

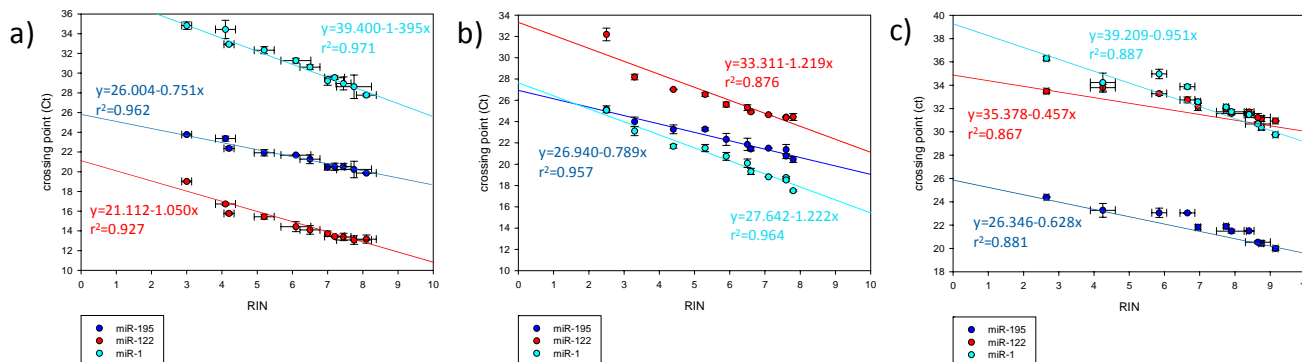
Results for miRNA quantification showed a highly significant increase in apparent miRNA percentage following ongoing RNA degradation for all investigated samples ($p < 0.001$) (figure 1). A significant rise in apparent miRNA concentration was demonstrated for liver and WBC samples. Gene expression was analysed for mRNA and miRNA. mRNA measurement showed a highly significant negative correlation for the crossing points (Ct) and the RNA integrity number (RIN) ($p < 0.001$), as it has also been shown by *Fleige et al. (2006)*¹.

Similar results could be obtained for miRNA gene expression (figure 2). A highly significant increase in the Ct with ongoing RNA degradation was shown ($p < 0.001$). This result demonstrates that miRNA is not as stable as it was thought of and is degraded in a similar manner as mRNA indicating the need for normalization also in miRNA gene expression analysis.

In conclusion, the determination of miRNA quantity with the Bioanalyzer is reliable for samples with good RNA quality. With increasing total RNA degradation level an overestimation of the miRNA amount occurs for miRNA quantification in the Bioanalyzer. The performance of miRNA qRT-PCR is dependent on the template quality as well as the performance of mRNA qRT-PCR.

Figure 2

Figure 2: miRNA gene expression data for a) liver, b) muscle, c) white blood cells (WBC); correlation between crossing point (Ct) and RNA integrity number (RIN) with $p < 0.001$ for all subsets



¹ Fleige S. & Pfaffl M.W.: RNA integrity and the effect on real-time qRT-PCR performance; *Molecular Aspects of Medicine*; 27 (2006); 126-139