The Impact of RNA Integrity on qRT-PCR Results

Fleige S. and Pfaffl M.W.
Physiology Weihenstephan, Center of Life and Food Sciences
TUM, Germany fleige@wzw.tum.de

Introduction:

An essential requirement for a successful quantitative mRNA analysis using qRT-PCR is the usage of intact RNA. Low-quality RNA may compromise the derived expression results. While it is obvious that intact RNA constitutes the best representation of the natural state of the transcriptome, there are situations in which gene expression analysis even on partially degraded RNA may be desirable.

Material and methods:

Total RNA was prepared from various bovine tissue samples. In order to get RNA samples with different and standardized degradation levels, intact cellular RNA was artificially degraded either enzymatic via ubiquitary skin RNAses or by irradiation with ultraviolet C radiation. Intact and degraded RNA samples from the identical tissue extraction were mixed in various ratios to generate a RIN gradient ranging from intact total RNA (RIN > 7.5) to highly degrade total RNA (RIN < 3). All RNA samples with different total RNA quality were investigated in triplicates in the Bioanalyzer (n = 405). In a second experiment seven PCR primer sets were designed to amplify different sequence fragments of ß-actin.

Results:

The dependency of the RNA quality on various tissue samples, white blood cells (WBC) and cell-lines were determined (table 1). The average RIN for solid tissues ranged between 5.4 and 9.6, whereas tissues or organs with high content of connecting tissues showed higher variations in RIN values. In cell culture and WBC the RIN ranged between 8.4 and 9.6 with low experimental variance.

Table 1. Tissue dependent RNA integrity

	Quality metrics			
tissue	*RIN*		*STABW*	*N*
lymph node	6.93	±	0.65	26
colon	7.52	±	0.62	19
corpus luteum	9.62	±	0.32	5
caecum	7.28	±	0.86	16
spleen	7.28	±	0.6	17
abomasum	7.30	±	0.86	17
WBC	9.63	±	0.13	5
kidney cells	8.87	±	0.32	3
granulosa cells	8.38	±	0.41	5
oviduct	9.40	±	0.29	5
reticulum	5.47	±	1.29	21
liver	6.49	±	0.86	28
ileum	7.35	±	1.53	17

Expression data demonstrate that a high-quality intact RNA (high RIN) determined a lower CP than by a less-quality RNA (low RIN) (figure1). Mean coefficient of determination in all regressed genes and tissues shows that there is a causally determined high correlation between RIN and the CP (P < 0.001).

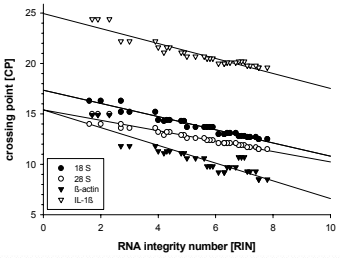

Fig. 1: Correlation between crossing point and RIN. Distribution of the RT-PCR absolute expression measured on 12 RNA aliquots in triplicates (n=36) from lymph node (cited as an example) using 18S, 28S, ß-actin and IL-1ß.

The RIN effect on qPCR efficiency was minor, compared to the influence on CP (figure 2).

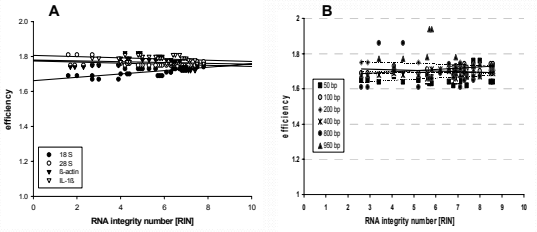

Figure 2: Effect on qPCR efficiency. A) Distribution of the RT-PCR absolute expression measured on 12 RNA aliquots in triplicates from lymph node using 18S, 28S, ß-actin and IL-1ß. B) Comparative analysis was done using ß-actin with different length of the amplified product

Relationship between RNA integrity and length of the amplified product

For all product length the crossing point is shifted towards lower cycle numbers using intact total RNA. The best repeatability could be attained in quadrant IV with a RIN value higher than five and a qRT-PCR product length lower as 200 bp.

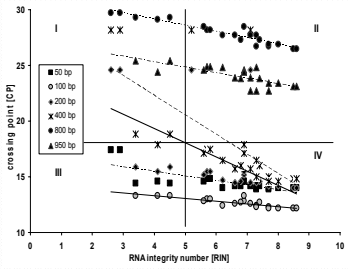

Fig. 2: Relationship between RNA integrity and length of the amplified product. Effect on the CP. Integrity of 23 bovine corpus luteum RNA sample profiles was scored using the RIN software. Comparative analysis was done using ß-actin with different length of the amplified product.

Discussion & Conclusion:

RNA quality control is indispensable prior to downstream quantification assays. Total RNA samples of high quality (RIN > 8) can serve as an optimal template whereas for partly degraded RNA (RIN > 5) results in sub-optimal qRT-PCR expressions. Degraded RNA interferes with PCR performance, expressed as CP value, whereas PCR efficiency is minor effected by RNA integrity. PCR efficiency seems to be major affected by the tissue type and extraction procedure.

References:

Fleige S and Pfaffl MW (2006): RNA integrity and the effect on the real-time qRT-PCR performance. Molecular Aspects of Medicine 27: 126–139.

Fleige S, Walf V, Huch S, Prgomet C, Sehm J and Pfaffl MW (2006): Comparison of relative mRNA quantification models and the impact of RNA integrity in quantitative real-time RT-PCR. Biotechnol Lett 28: 1601–1613.