

New tools for quality control in real-time PCR experiments

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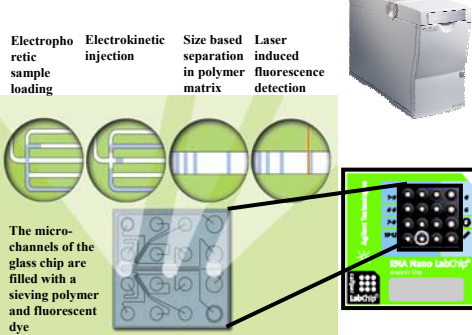
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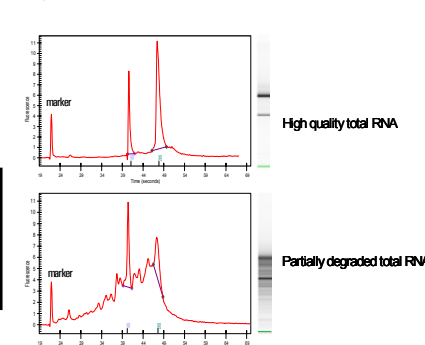
Abstract

The reliability of the real-time PCR data is largely dependent on the quality of the assay itself as well as on the quality of the template and the specificity of the PCR products. Lab-on-a-Chip technology allows the quality assessment of RNA and PCR samples at different steps within the real-time PCR workflow. Applied on an analytical platform, the degradation state of RNA samples can be monitored allowing RNA integrity assessment at the beginning of an experiment. To improve the standardization of the RNA QC process, a software algorithm has been developed for the classification of RNA samples. First test indicated that analyses by the software are more reliable than the manual evaluation of RNA integrity by considering the ribosomal ratio of the samples. Furthermore, in addition to melting curve analysis, the high resolution of the Lab-on-a-chip-technology provides the opportunity to verify the specificity of PCR products at the end of an experiment. In summary, here we provide data how quality control can be implemented in a real-time PCR study to improve the reproducibility and reliability of gene expression experiments.

Separation principle



Degradation of Total RNA



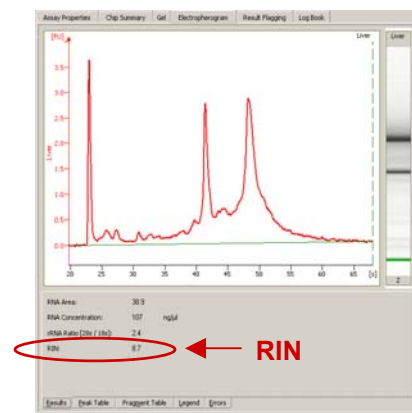
Introducing RIN

- software algorithm that allows classification of RNA integrity
- extracts a number of characteristic features from the bioanalyzer electropherogram
- Uses adaptive learning process to "teach" the algorithm about the relative importance of the extracted features
- returns a value that is characteristic for the integrity of a specific sample.

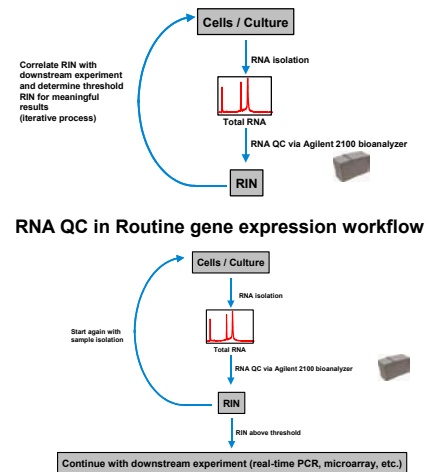
What the RIN can do:

- Obtain an assessment of RNA integrity.
- Directly compare RNA samples
- Ensure repeatability of experiments

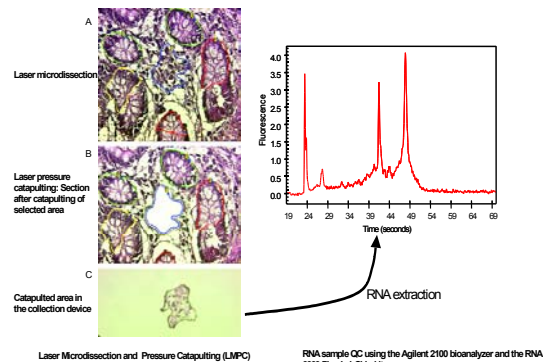
RNA Integrity Number (RIN) – A standardized approach for RNA integrity assessment



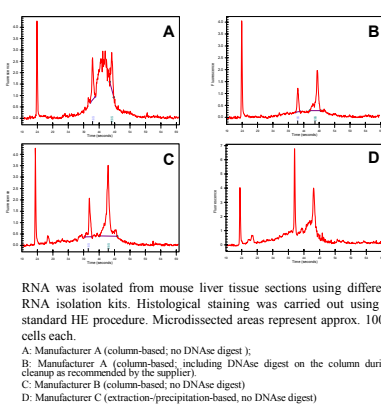
RIN Validation for Gene Expression Experiment



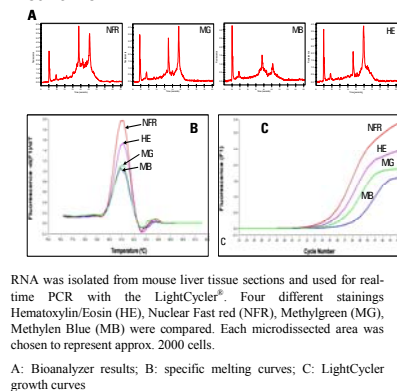
RNA Quality Control of microdissected samples



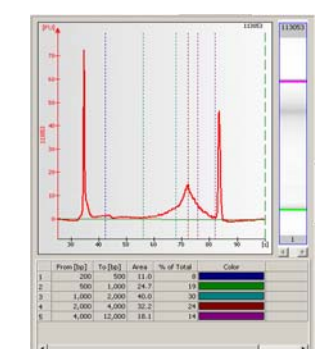
Variability in RNA Isolation Efficiency



Correlation of Bioanalyzer RNA QC Results to Real-time PCR

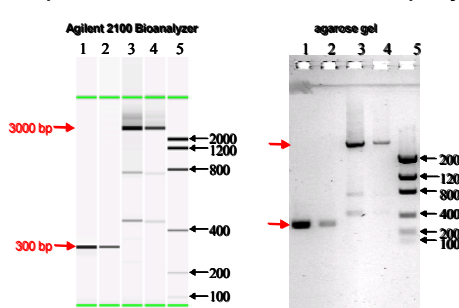


Comparative cDNA analysis



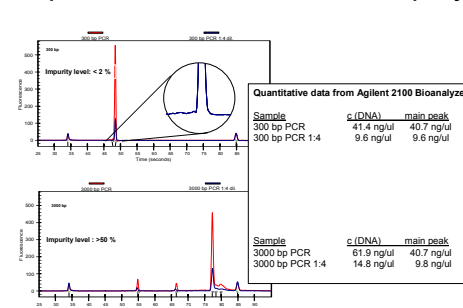
Complex cDNA samples can be analyzed by using smear analysis. The software allows to define regions of interest. These regions are used to define the area of broad peaks and determine their part of the total area. Smear analysis provide a means to analyze broad signals that can be hardly evaluated with the normal peak assignment.

Improved Determination of PCR Product Impurity



Comparison between the analysis of two PCR reactions (300 and 3000 bp products) using the lab-on-a-chip technology vs. an agarose gel. Two different concentrations are shown side by side for each PCR reaction. The analyses of the 300bp fragment are shown in lane 1 (undiluted) and lane 2: (1:4 dilution). Whereas the 3000 bp fragment was analyzed on lane 3 (undiluted) and lane 4 (1:4 dilution).

Improved Determination of PCR Product Impurity



The quantitative data generated using the bioanalyzer indicates the amount of impurity or non-specific products in these PCR reactions. The electropherogram shows a pure 300 bp product vs. the 3000 bp product with several non-specific bands that can be quantitated.