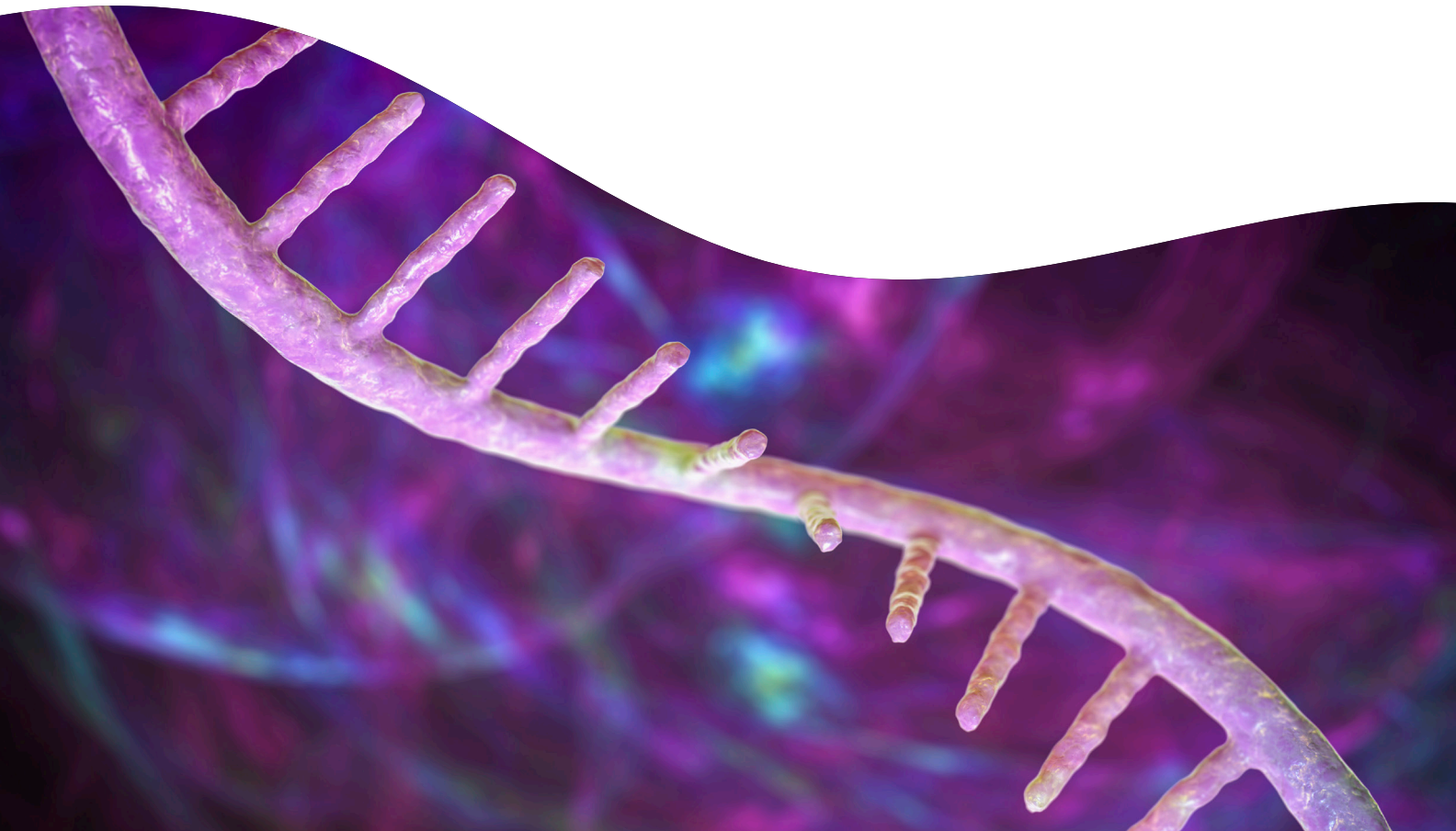
APPLICATION NOTE

RNA quality score (RQS) calculation and correlation to RIN.

RNA quality and integrity are predictive of the likelihood of success for downstream gene expression experiments such as microarray analysis or real-time PCR. The LabChip™ GX Touch™ Nucleic acid Analyzer analyzes RNA by electrophoretic separation on microfluidic sipper chips. RNA molecules are separated and subsequently detected via laser induced fluorescence. The LabChip GX Software displays the raw data as an electropherogram and generates a gel-like image for visualization, data such as peak heights, peak areas, concentration, etc., are then calculated and stored as digitized data as text files and tables. These parameters are next used to determine an RQS (RNA Quality Score) number, a calculated score that rates the quality of RNA samples. The RQS correlates well with Agilent's RIN (RNA Integrity Number) and follows the same 0-10 scale rating. Results comparing RIN to RQS for the same samples run on both LabChip GX Touch system and Agilent's Bioanalyzer 2100 typically show <10% deviation. The RQS is consistent over the LabChip standard sensitivity RNA assay concentration range (25 to 250 ng/µL) with CV's <20%.



RQS Validation

The method used to develop the RQS was done by testing a variety of different RNA tissue types that underwent two different methods of degradation, heat or RNase. Total RNA was isolated from 8 different tissue types (rat brain, rat liver, human kidney, mouse ovary, HeLa, Raji, Jurkat, and mouse heart) and were degraded by heating at 90°C for various time points (0, 5, 10, 15, 17.5, 20, 22.5, 25, 30, 40, 50 minutes). Two (2) µL of the heat degraded samples were transferred to a 96 well plate and analyzed on the LabChip GX Touch system.

Figure 1 shows a typical electropherogram from a partially degraded RNA sample. The calculation of the RQS factors in the 18S and 28S peak areas and heights, as well as the total RNA area. It also uses the FastRegion Area (region between the LM and 18S peaks), which is representative of smaller RNA fragments and presumably degraded products. The figure shows the areas considered in the calculation of the RQS.

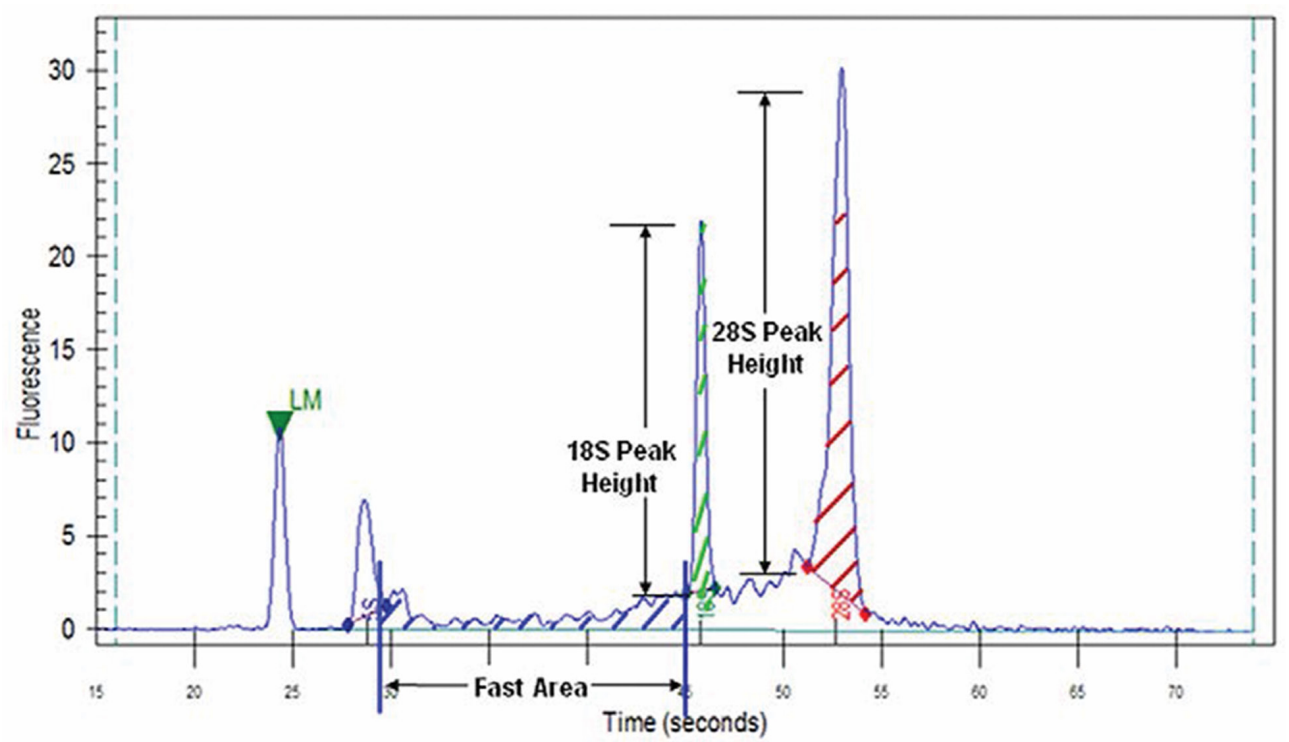


Figure 1: The RQS calculation factors in the 18S and 28S peak areas and heights, as well as the total RNA area. In addition, the FastRegion Area (region between the Lower Marker and 18S peaks) is used, which is representative of smaller RNA fragments and presumably degraded products.

$$QS = A + \left(1 - \frac{FastRegionArea}{TotalArea}\right) * X_1 + \left(\frac{18SArea + 28SArea}{TotalArea}\right) * X_2 + \left(\frac{28SHeight}{18SHeight}\right) * X_3$$

A, X1, X2, and X3 are constants

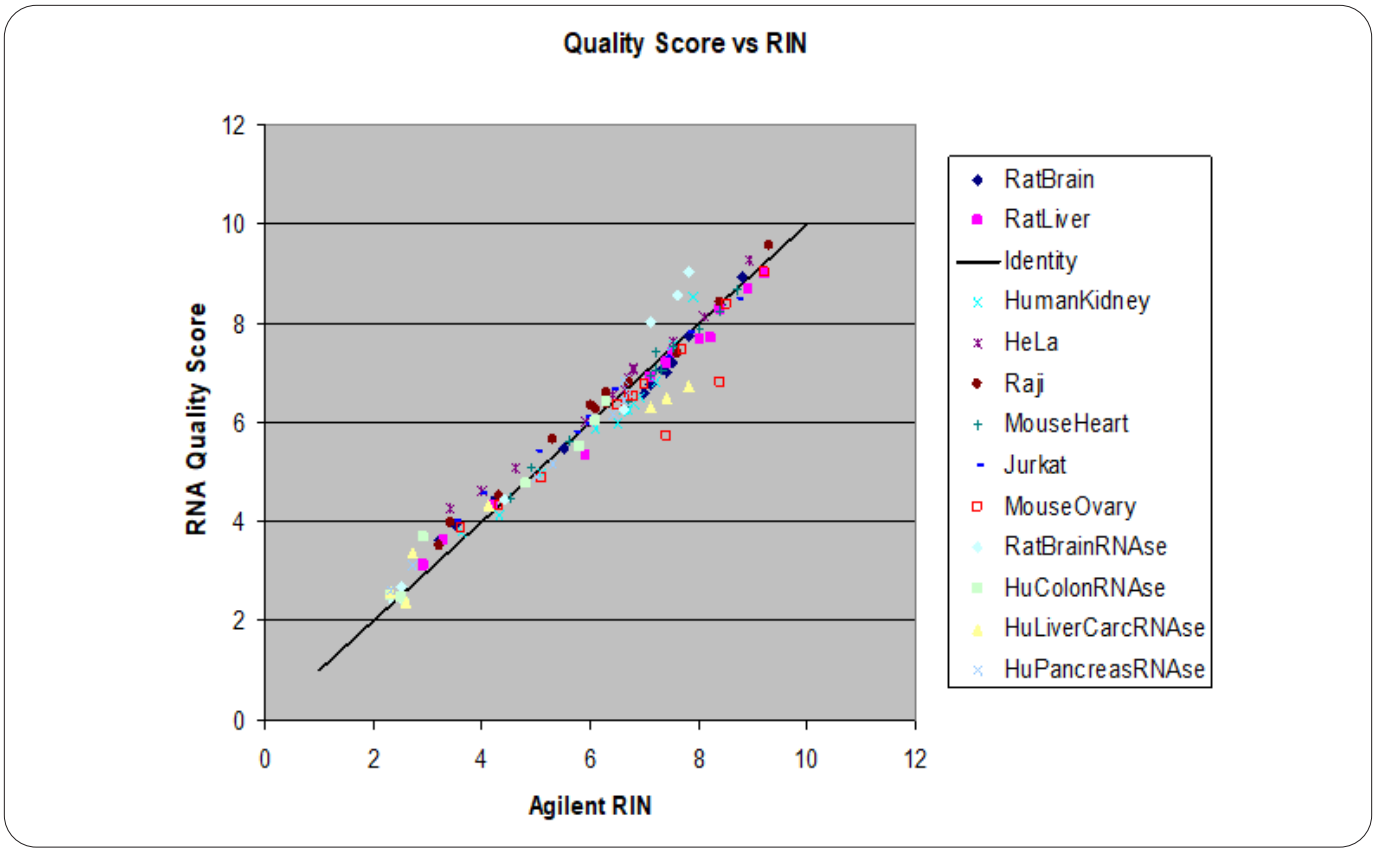
RQS equation

RQS Reproducability

RQS reproducibility was determined by analyzing total RNA samples derived from 4 different tissue types. Three different concentrations (50, 100, 200 ng/µL) of each intact total RNA were degraded with 8 levels of RNase digestion. All reactions were run in triplicate. The results showed <10% CV across the replicates and tissue types. Constancy of RQS scoring was also tested on 320 replicates of intact rat brain total RNA at 25, 50, 100, 250, 500 ng/µL. The samples were run on 4 different chips across 4 different LabChip GX Touch systems. The resulting population mean was RQS = 8.41 with a CV of 13.5%. RNA concentrations below 12.5 ng/µL (below the concentration spec for the assay) were shown to be unreliable and RQS scores are not reported in the data tables.

RQS vs RIN

The RNA Integrity Number (RIN) was developed by Agilent to help scientists estimate the integrity of total RNA samples and has been considered the standard for determining RNA quality. Correlation of the LabChip RQS to the Agilent RIN was determined by running a total of 96 heat degraded samples on both the Agilent BioAnalyzer 2100 and the LabChip GX Touch system. 4 different tissue types (rat brain, human pancreas, human colon, and human liver carcinoma) were degraded with an RNase cocktail (Thermo Scientific) at different concentrations (1,600x, 3,200x, 6,400x, 12,800x, 25,600x, 51,200x, 102,400x). After 2 minutes of incubation, 2 µL of 0.1x SUPERaseIn™ (Thermo Scientific) was added to the reactions to stop the degradation process. 2 µL of the samples were transferred to a 96 well plate and analyzed on the LabChip GX Touch system, while 1 µL of the same sample was analyzed on the Agilent 2100 Bioanalyzer. Heat denatured samples (method described above) were also tested. The correlation curve for the RQS vs RIN for 156 samples is shown below.



Summary

The LabChip GX Touch System for RNA analysis

- Provides a single number rating of RNA quality that can qualify RNA samples prior to use in gene expression studies.
- RQS correlates well to Agilent's RIN – Typically <10% deviation from RIN for the same sample run on Bioanalyzer and LabChip GX Touch system.
- RQS is consistent over whole concentration range (25 to 250 ng/µL).
- RQS is consistent for RNA samples collected from a wide variety of different tissues.
- RQS is consistent for RNA that may have degraded by different mechanisms.
- RQS reproducibility: CV < 20% over whole concentration range and wide range of degradation.