

## 1 RNA Assessment

RNA quality and integrity analysis provide predictive metrics for the downstream success of gene expression experiments, such as next generation sequencing, microarray analysis and real-time PCR. The universally accepted metric for RNA quality assessment is the Agilent RIN (RNA Integrity Number) score. We have created a similar quality RNA Quality Score (RQS) to determine the quality of RNA samples, which shows a high correlation to RIN.

A new metric has recently been promoted by Illumina Corporation called DV<sub>200</sub>. It was developed to more accurately reflect RNA quality from degraded FFPE samples required for successful downstream next-generation sequencing library preparation.

Herein, we evaluate our RQS calculation to the RIN score as an acceptable measure for RNA quality. Additionally, we explore our LabChip<sup>®</sup> GX Touch DV<sub>200</sub> metric calculation as it compares to alternative instruments. We demonstrate statistically comparable results to the RIN score, and nearly identical DV<sub>200</sub> scores to alternative instruments.

### LabChip<sup>®</sup> GX Touch for Genomic Applications

- Automated analysis of RNA samples in ~80 seconds
- Automatic calculation of RNA Quality Score (RQS) - statically comparable to RIN
- Flexible data display formats - virtual gel, electropherogram or tabular



Figure 1. LabChip GXII Touch Instrument

Automated electrophoretic separation of DNA and RNA. Multiple assays available equal to standard agarose separation densities.

## 2 RNA Quality Score Calculation

The RNA Quality Score (RQS) calculation was designed to provide an equivalent metric to the RIN score associated with Agilent Technologies, Inc. The figure below illustrates a typical electropherogram from a partially degraded RNA sample with the areas considered for the RQS calculation.

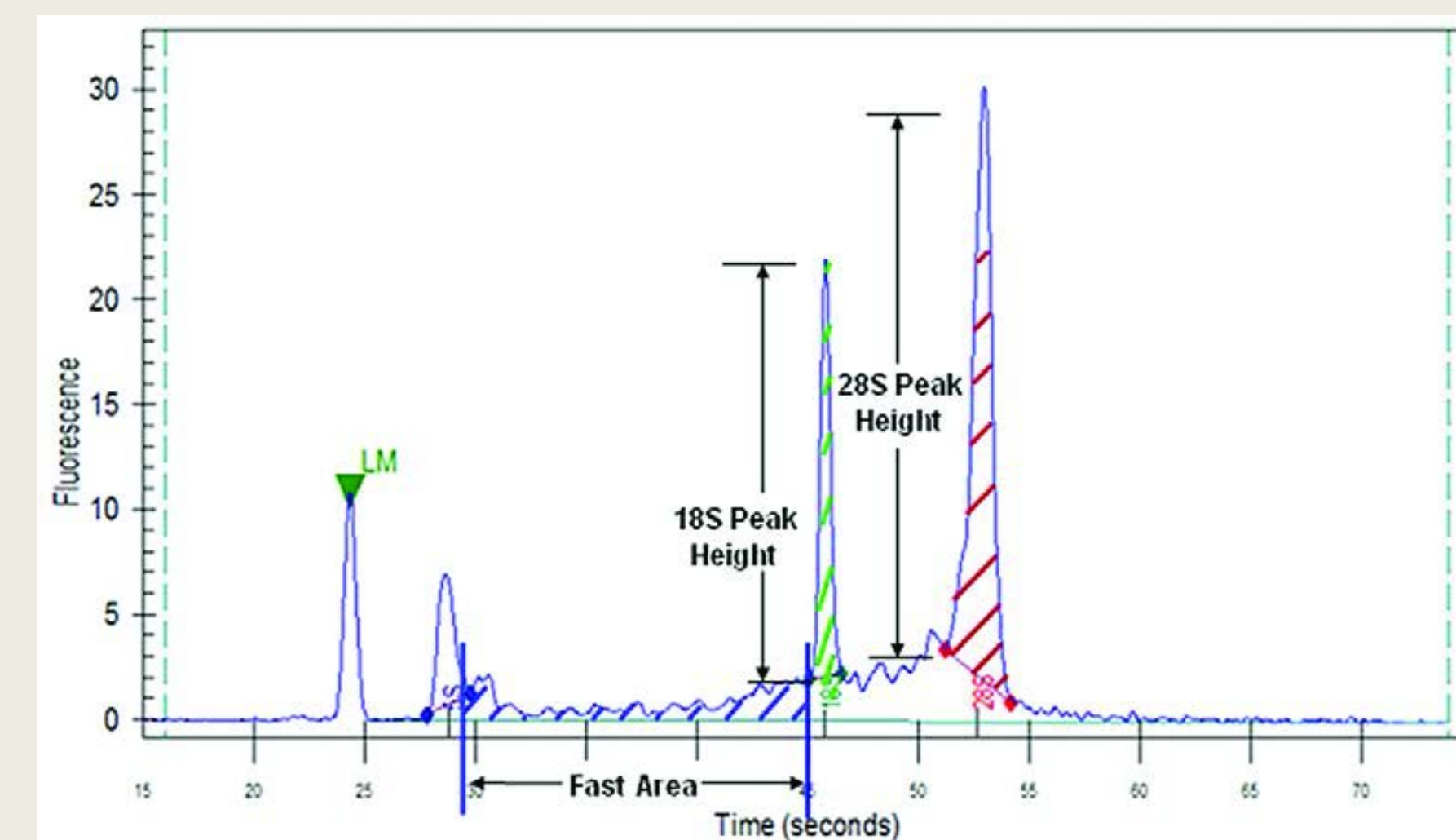


Figure 2. The RQS calculation factors in the 18S and 28S peak areas and heights, as well as the total RNA area. In addition, the FastRegion Area (region between the Lower Marker and 18S peaks) is used, which is representative of smaller RNA fragments and presumably degraded products.

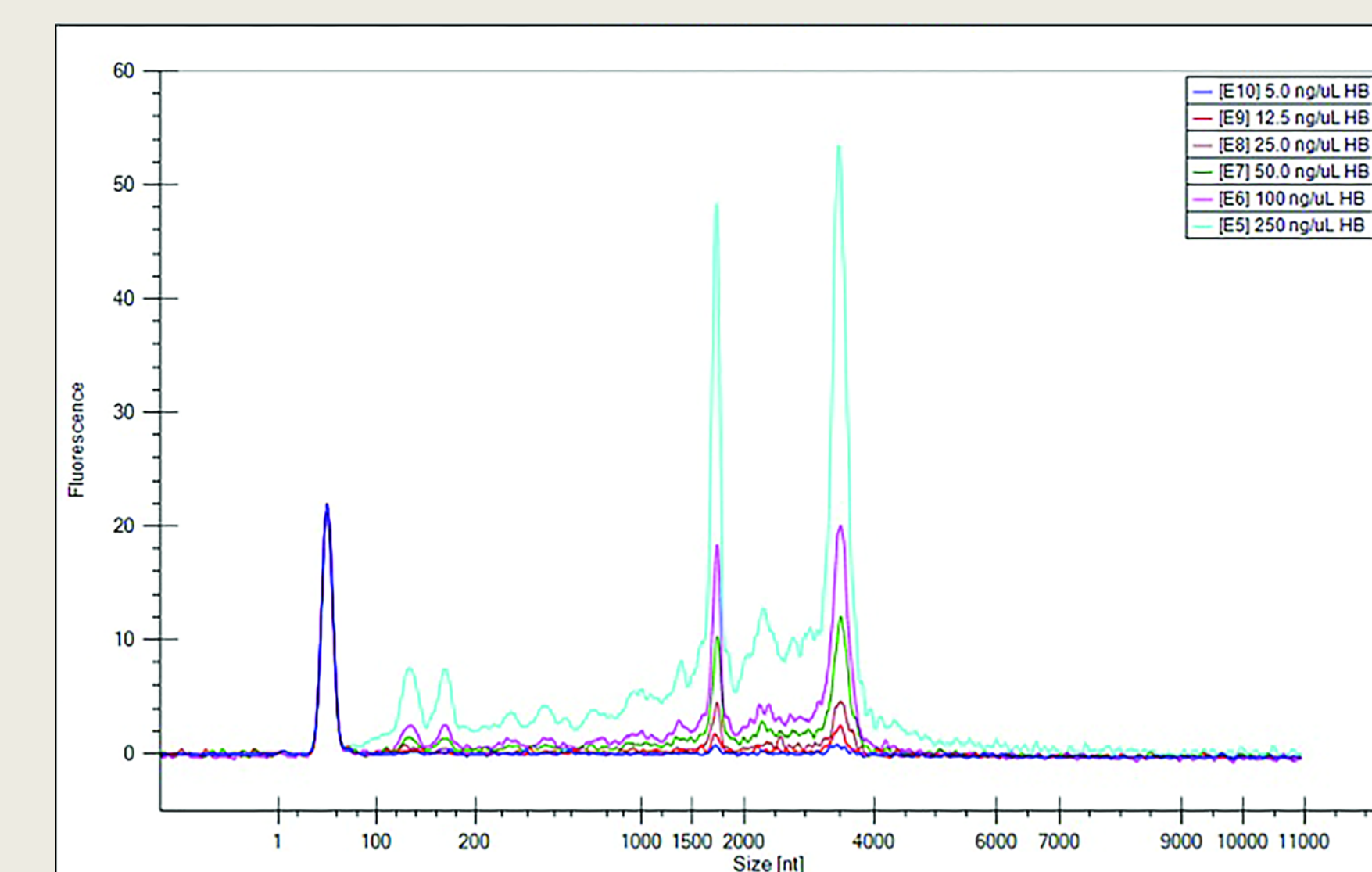
RQS is Defined by the Equation

$$RQS = A + \left(1 - \frac{\text{FastRegionArea}}{\text{TotalArea}}\right) * X_1 + \left(\frac{18S\text{Area} + 28S\text{Area}}{\text{TotalArea}}\right) * X_2 + \left(\frac{28S\text{Height}}{18S\text{Height}}\right) * X_3$$

A, X1, X2, and X3 are constants

## 3 RQS Reproducibility

Human Universal RNA at 500 ng/μL and Human Brain RNA at various concentrations (250, 100, 50, 25 ng/μL) were run in 8 repeats. The results showed in Figure 3 are <10% CV across the replicates and tissue types.



RNA Sample (ng/μL)	Avg. RQS Score	CV
Human Universal 500	8.60	1.48 %
Human Brain 250	7.61	1.20 %
Human Brain 100	7.70	0.76 %
Human Brain 50	7.73	1.15 %
Human Brain 25	7.81	0.78 %
Human Brain (avg.)	7.71	1.10 %

## 4 RQS Comparison to RIN Score

To validate the RQS metric, we tested ten different mammalian RNA tissue types under two different methods of degradation, as well as concentration titration on one Human RNA (human heart) and one Rat RNA (Rat liver). The correlation curve for the RQS vs RIN is shown below.

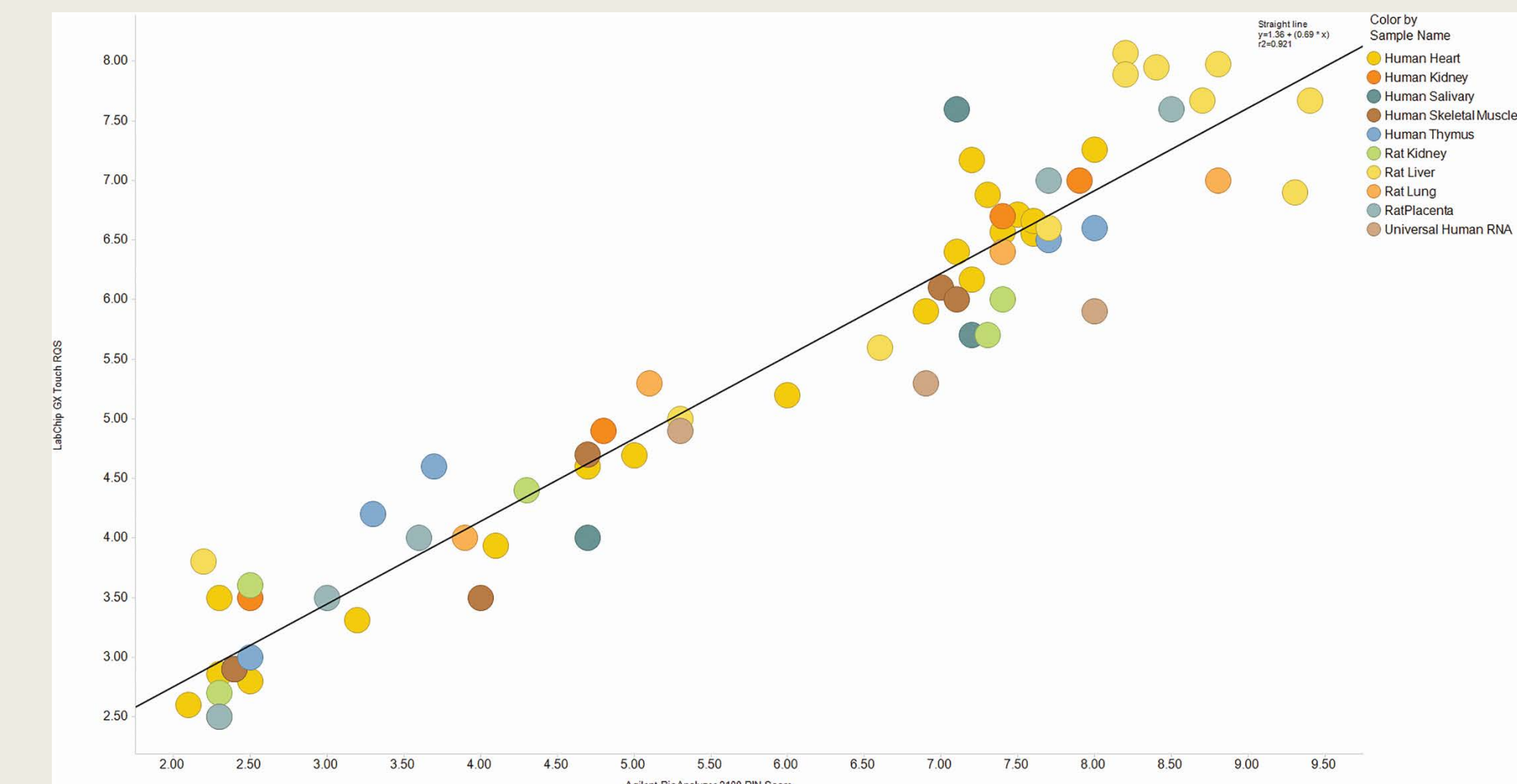


Figure 4. Commercially available RNA from 10 different tissues types (final concentration 100 ng/μL) and degraded by either heat treatment at 90°C for various time points (0, 5, 10, 15, 20, 30, 45, 60 minutes) or RNase I digestion (0x and 25600x concentrations). Titration concentrations are 250, 200, 150, 100, 75, 50, 25 and 0 ng/μL. R<sup>2</sup>=0.921

## 5 DV<sub>200</sub> RNA Quality Metric

A new metric has recently been promoted by Illumina Corporation called the DV<sub>200</sub> (the percentage of RNA fragments > 200 nucleotides) to more accurately predict downstream performance of RNA samples. As an alternative to the RIN score, it serves as a measure of RNA quality and has been shown to be a reliable predictor of successful library preparation, especially for FFPE samples.

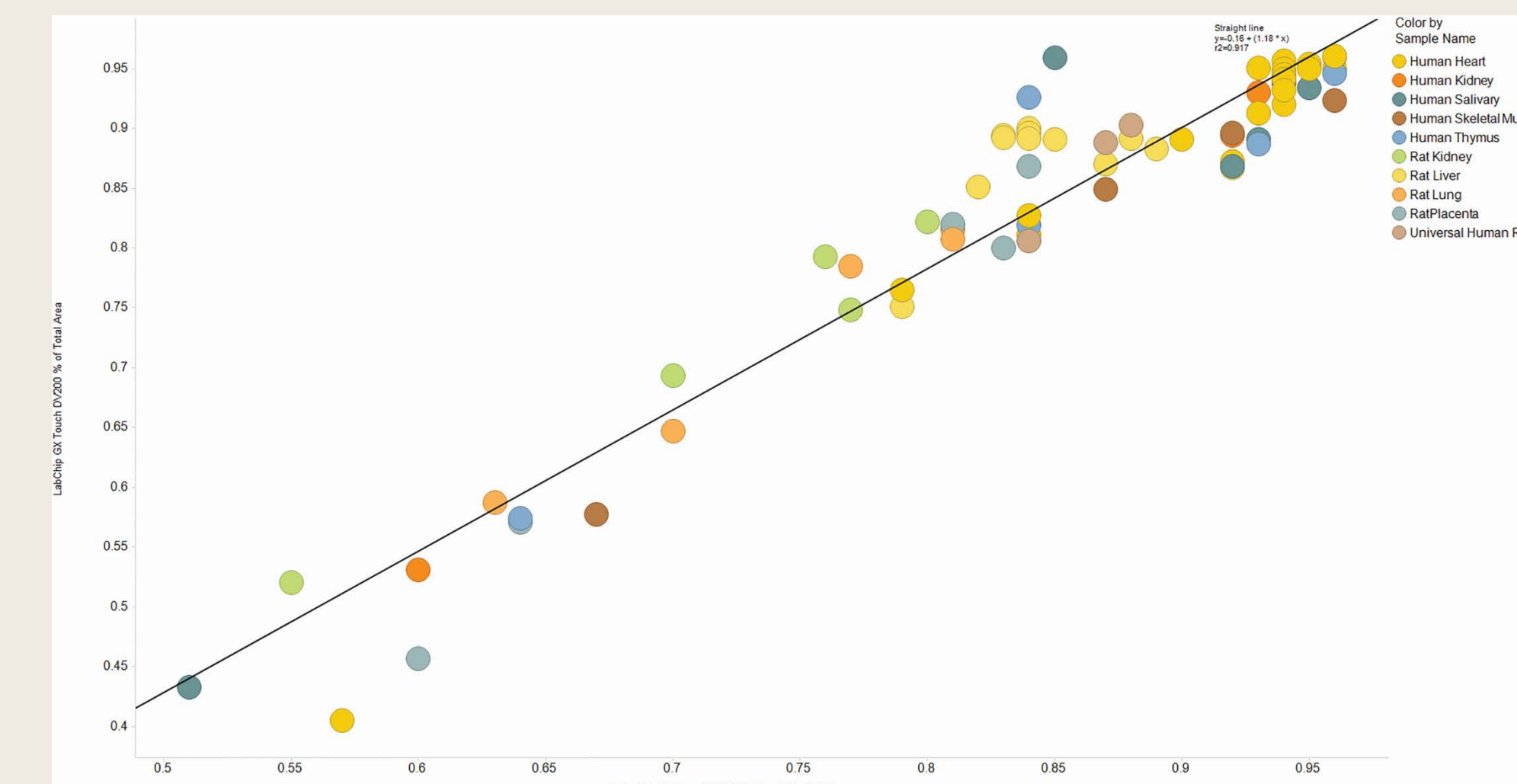


Figure 5. Analysis settings in the LabChip GX reviewer software can easily be adjusted to calculate DV<sub>200</sub> values, and these settings can be applied automatically during the run. The figure above illustrates a high correlation of DV<sub>200</sub> values between LabChip GX Touch and Agilent BioAnalyzer 2100 platforms. R<sup>2</sup>=0.917

## 6 LabChip GX Touch Reported Concentration

LabChip GX Touch HT RNA Assay quantitation was validated with ladder as sample. Here, we present that intact RNA samples between 25-250 ng/μL. All reported concentration are within the 30% concentration accuracy specification. This specification may also apply to degraded samples depends on sample type and degradation method.

RNA Sample	Expected Concentration (ng/μL)	LabChip GX Touch Reported Concentration (ng/μL)
Human Brain	25	25.69
Human Brain	50	61.03
Human Brain	100	101.83
Human Brain	250	281.85
Rat Liver	25	25.16
Rat Liver	50	51.69
Rat Liver	75	77.08
Rat Liver	100	106.94
Rat Liver	150	161.32
Rat Liver	200	215.32
Rat Liver	250	287.14

## 7 Ideal Solution for High Throughput RNA Quality and Assessment

The LabChip GX Touch Instrument automates electrophoretic separation for DNA and RNA. Here, we demonstrate its use for RNA quality analysis with comparisons to the universally accepted RIN score and DV<sub>200</sub> metric over a 25-250 ng/μL concentration range with a wide range of degradation.

RQS score reported by LabChip GX Touch is statically comparable to the RIN score.

LabChip GX software reports a DV<sub>200</sub> calculation similar to that of the Agilent BioAnalyzer 2100 platform.

LabChip GX Touch HT RNA Assay Specifications	
Linear Range	25 to 250 ng/μL (high range) 5 to 50 ng/μL (low range) 500 to 5,000 pg/μL (with Pico RNA Reagent Kit)
Quantification Reproducibility	<20% CV (from chip to chip and instrument to instrument)
Quantification Accuracy	< +/-30% error with ladder as sample
Size Range	100 to 6,000 nucleotides (suitable for total RNA)
RNA Sample Volume	2 μL of user sample for high range assay 6 μL of user sample for low range assay
Run Time	80 sec. per sample (about 2.5 hours for 96-well plate)
Setup Time	About ½ hour for chip and sample prep
Number of Samples per Chip Prep	200 samples max, then dye/gel must be replaced
Reagent Kit Lifetime	Up to 5 chip primes (typical modeled workflow is 96 samples per prime, thus 480 samples per reagent kit)  Some high volume users may realize the full 200 sample capacity per prime → 1,000 samples per reagent kit.
Chip Lifetime	>2,000 samples