DV_{200} RNA quality metric for FFPE analysis using LabChip GX Touch nucleic acid analyzer.

The process of fixing the tissue sample and embedding it in paraffin can cause severe degradation of the RNA. The fixation process and storage cause significant RNA degradation in some samples. The formalin fixation process causes cross linkage between nucleic acids and proteins, and the covalent modification of RNA by mono-methylol ($-CH_2OH$) addition to the bases[1]. Thus, it is very important to assess RNA quality after FFPE (Formalin-Fixed, Paraffin-Embedded) extraction of RNA for downstream applications.

One measure of RNA quality is the RNA Quality Score, or 'RQS'. The RQS is a metric calculated by the LabChip™ RNA Assay and has a high degree of correlation to the Agilent RNA Integrity Number, or 'RIN'. While these are the most prominent available RNA metrics, they may not apply equally to all types of RNA applications.

The DV_{200} metric has been promoted by Illumina®. As noted in the Illumina tech note[2] "Many researchers use the Agilent RNA Integrity Number (RIN) to determine RNA quality for gene expression analysis. However, we have found that RIN values from degraded FFPE samples are not a sensitive measure of RNA quality nor are they a reliable predictor of successful library preparation".

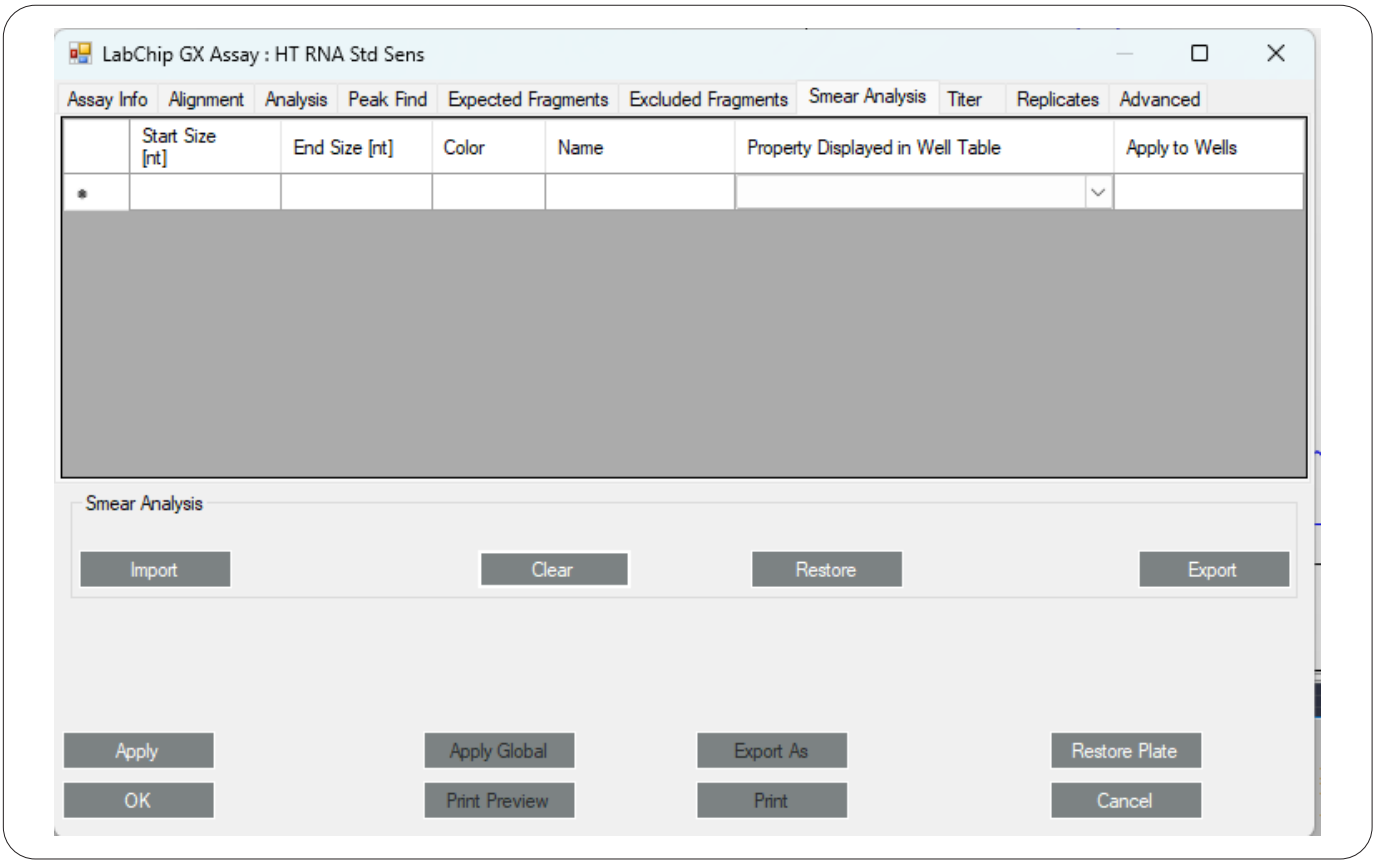
To improve the RNA integrity assessment, Illumina developed the DV_{200} metric – the percentage of RNA fragments > 200 nucleotides to assess FFPE RNA quality. Following is the calculation of DV_{200} on the LabChip™ GX Touch™ nucleic acid analyzer.

Evaluation of FFPE Samples for RNA Quality on LabChip GX Touch

Here we show how to obtain the DV_{200} value by analyzing RNA FFPE samples on the LabChip GX Touch system. Prior to the DV_{200} metric, the LabChip GX Touch software calculated a RQS (RNA Quality Score) value. It has been well demonstrated that there is a high correlation between the RQS value and Agilent RIN value for a large number of sample types, regardless of denaturation method used[3].

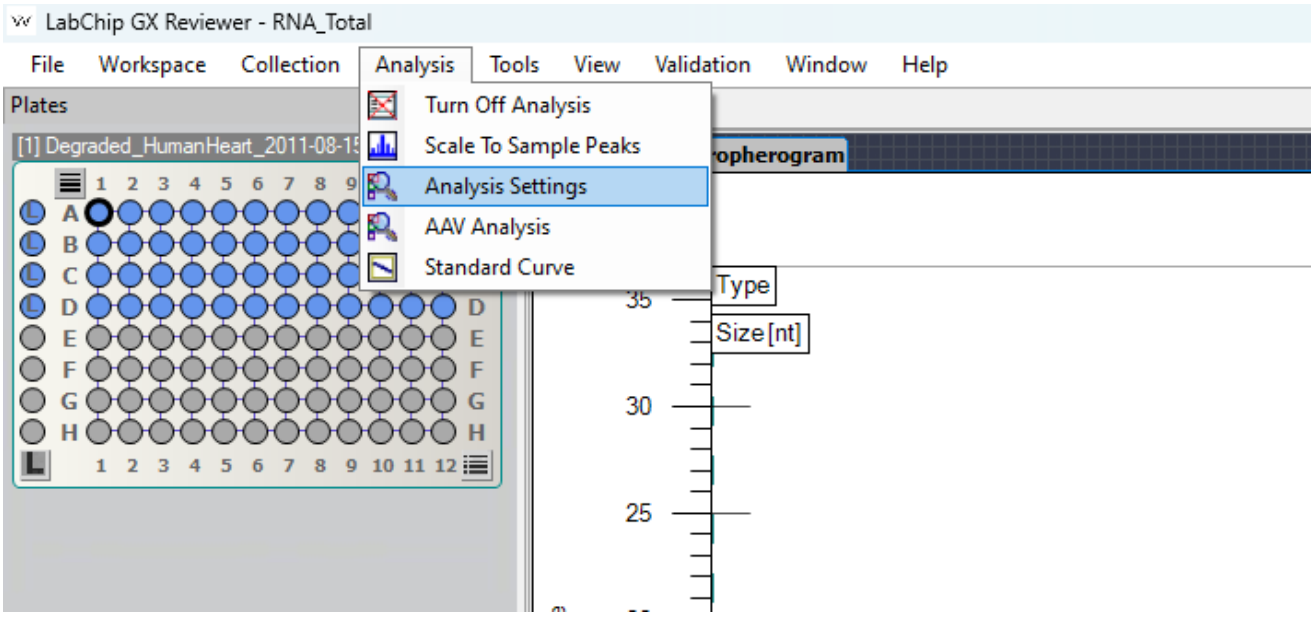
In addition to the RQS value, the LabChip Reviewer software can now calculate the DV_{200} value. Both values can be exported and utilized to determine the RNA quality of FFPE samples.

1. Run samples on the LabChip GX Touch instrument. For further information on sample preparation and running the samples, refer to RNA Assay User Guide(s).
2. Open data files in the LabChip GX Reviewer Software.
3. Select 'Analysis Settings' under 'Analysis' tab.

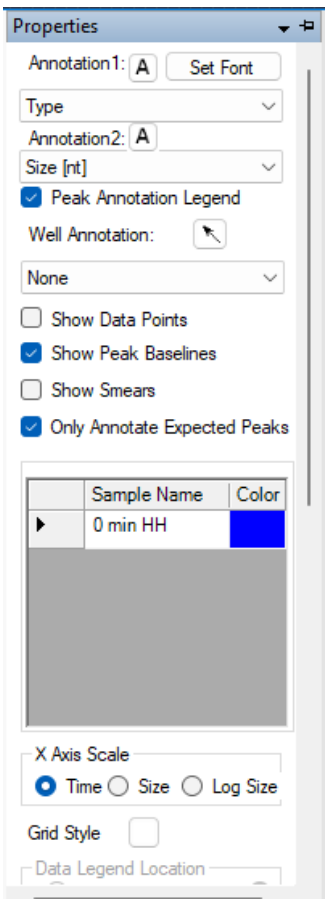


4. Select 'Smear Analysis' tab.
5. Define region from 200 to 10000 nt. Enter the size were region starts under Start Size. Add the size were region ends under End Size
6. Under Name column, type in DV_{200} or give any other name to the value being reported.
7. Select '% of Total Area' from the drop-down menu under 'Property Displayed' in Well Table.
8. Analysis can be applied to all or select samples under 'Apply to Wells' tab.
9. Click OK.

10. If multiple plates have been analyzed click 'Apply Global'.



11. To highlight the selected region in the electropherograms and gel image check 'Show Smears' in the properties.



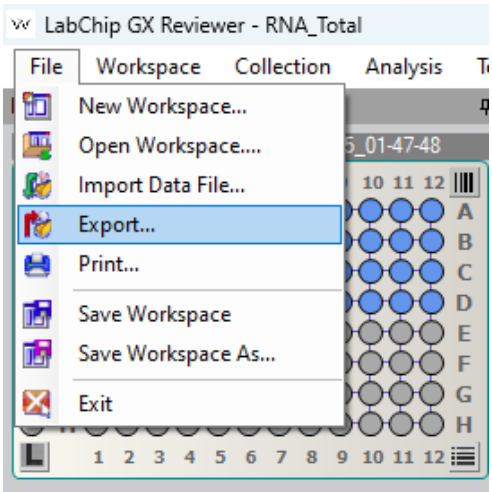
12. Select Well Table within LabChip GX Reviewer software (Figure 1).

Well Table | Peak Table | Filter | Gel

	Plate Name	Sample Name	Well Label	Total Conc. (ng/ul)	RNA Area	rRNA Area Ratio [28S/18S]	rRNA Height Ratio [28S/18S]	rRNA Fast Area	RNA Quality Score	5S Area	5S %Total	18S Area	18S %Total	28S Area	28S %Total	DV200 % of Total
▶	Degraded_HumanHeart_2...	Ladder1	Ladder01	2.000	291.79											
	Degraded_HumanHeart_2...	0 min HH	A01	1.812	261.98	2.84	1.42	0.23	7.1	7.52	2.9%	16.03	6.1%	45.57	17.4%	84.6%
	Degraded_HumanHeart_2...	5 min HH	A02	1.951	266.62	1.32	0.76	0.51	5.0	8.24	3.1%	16.06	6.0%	21.17	7.9%	75.8%
	Degraded_HumanHeart_2...	15 min HH	A03	2.081	269.82	1.13	0.72	0.52	4.8	7.86	2.9%	13.65	5.1%	15.47	5.7%	75.4%
	Degraded_HumanHeart_2...	30 min HH	A04	1.922	270.46	0.94	0.62	0.53	4.7	8.12	3.0%	14.26	5.3%	13.36	4.9%	75.2%
	Degraded_HumanHeart_2...	45 min HH	A05	1.889	264.15	0.52	0.49	0.55	4.4	8.14	3.1%	13.12	5.0%	6.83	2.6%	74.6%
	Degraded_HumanHeart_2...	60 min HH	A06	2.130	266.61	0.42	0.41	0.57	4.3	8.02	3.0%	11.94	4.5%	4.97	1.9%	74.5%
	Degraded_HumanHeart_2...	75 min HH	A07	1.926	264.48	0.26	0.33	0.60	4.0	21.60	8.2%	9.18	3.5%	2.40	0.9%	73.9%
	Degraded_HumanHeart_2...	90 min HH	A08	1.929	257.69	0.06	0.23	0.59	4.0	7.97	3.1%	9.86	3.8%	0.59	0.2%	73.8%
	Degraded_HumanHeart_2...	0 min HH	A09	1.856	261.28	2.70	1.38	0.24	7.0	15.06	5.8%	16.16	6.2%	43.59	16.7%	84.6%
	Degraded_HumanHeart_2...	5 min HH	A10	2.094	265.00	1.08	0.77	0.51	5.0	8.15	3.1%	15.83	6.0%	17.14	6.5%	75.6%
	Degraded_HumanHeart_2...	15 min HH	A11	1.952	261.74	1.10	0.68	0.52	4.9	7.60	2.9%	15.26	5.8%	16.79	6.4%	75.3%
	Degraded_HumanHeart_2...	30 min HH	A12	1.928	262.39	0.60	0.58	0.53	4.6	7.85	3.0%	13.69	5.2%	8.27	3.2%	75.0%
	Degraded_HumanHeart_2...	Ladder2	Ladder02	2.000	282.49											
	Degraded_HumanHeart_2...	45 min HH	B01	1.998	260.11	0.82	0.51	0.57	4.3	7.78	3.0%	10.34	4.0%	8.51	3.3%	74.8%
	Degraded_HumanHeart_2...	60 min HH	B02	2.170	260.93	0.38	0.36	0.56	4.3	7.28	2.8%	11.31	4.3%	4.27	1.6%	74.7%
	Degraded_HumanHeart_2...	75 min HH	B03	2.055	260.38	0.35	0.25	0.59	4.0	7.44	2.9%	9.62	3.7%	3.33	1.3%	74.2%
	Degraded_HumanHeart_2...	Ladder3	Ladder03	2.000	280.18											
	Degraded_HumanHeart_2...	Ladder4	Ladder04	2.000	270.00											

Example of data table (fragment) with RQS and DV200 values calculated.

13. Results can be exported to Microsoft® Excel, standard LIMS or other types of databases for further analysis. Select 'Export' under 'File' tab and follow the instructions.



References

1. Masuda, N. et al., (1999) Nucleic Acids Res. 27(22), 4436.
2. Evaluating RNA Quality from FFPE Samples, Tech Note: RNA sequencing, Illumina. Pub No. 470-2014-001, April 15 2014.
3. RNA Quality Score (RQS) Calculation and Correlation to RIN, Applications Note 402 Caliper Life Sciences Nov 2009.