LabChip Microfluidics

Key Features:

- Automated analysis of RNA in about 80 sec
- Significant cost savings compared to manual gels
- Flexible data display options — virtual gel, electropherogram graph or tabular formats
- Automatic calculation of sample quality score (RQS)
- Automatic calculation of sample Quality Score of DV_{200}

Automated Integrity Assessment of RNA Samples

Introduction

LabChip® RNA analysis provides a fast and easy way to quantitate RNA samples ranging in size from 100 to 6,000 nucleotides. Performing RNA sample analysis with the RNA assay saves time and money by automating steps for quantification and integrity analysis. LabChip RNA analysis provides the user with RNA concentration and ribosomal ratios as an indicator of integrity.

A single sipper microfluidic chip is used to aspirate RNA samples directly from 96- or 384-well plates. The microfluidics chip technology automatically mixes in an intercalating dye, electrophoretically separates and analyzes the RNA sample. The instrument optics detect the laser-induced fluorescent signal. System software automatically analyzes the data and determines fragment size and concentration using ladder and marker calibration standards. Digital data results are immediately available for review or reporting in virtual gel, electropherogram graph or table summary form.

Figure 1. The LabChip GX Touch platform offers rapid RNA quantification.

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For the Better

RNA Quality Score (RQS)

The RQS determines the degree of sample degradation over a wide range of RNA sample types and experimental conditions. This scoring system takes into account many different characteristics of the sample electropherogram, such as the Fast Region Area percentage, the 18S and 28S area percentage, and the ribosomal ratio of 28S to 18S peak height (Figures 2 and 3). The result is a Quality Score that ranges between '0' and '10', where '10' is an intact sample. This score can be used as a standalone evaluation, or used in conjunction with the individual parameters that GX Touch software provides for quantifying RNA samples. The RQS correlates extremely well to other commercially available indices.
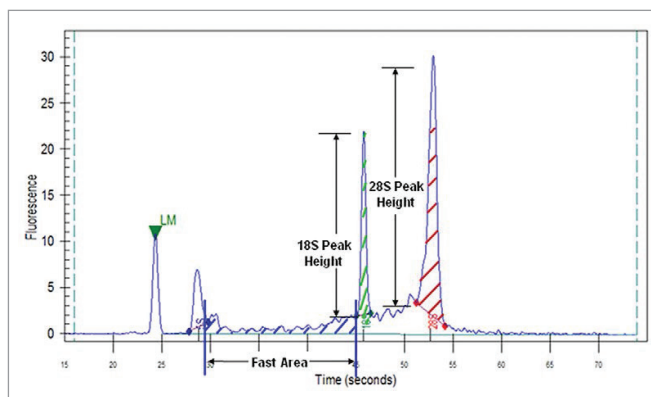

Figure 2. The RQS scoring system takes into account many different characteristics of the sample electropherogram.

Example of Quantitative Analysis Used to Detect Degraded RNA Samples

Well Name	RNA Frag	Height	rRNA Height Ratio	rRNA Area Ratio	% of total Area	Fast Area Ratio
Intact 100	18S	9.7	1.91	3.15	13.3	-0.02
	28S	18.6	1.91	3.15	41.9	-0.02
RNA 10	18S	19.1	1.07	2.55	10.4	0.12
	28S	20.5	1.07	2.55	26.5	0.12
RNA 20	18S	16.9	0.78	2.21	8.6	0.24
	28S	13.1	0.78	2.21	18.9	0.24
RNA 30	18S	15	0.58	1.21	7.8	0.23
	28S	8.7	0.58	1.21	9.5	0.23
RNA 40	18S	10.7	0.4	0.97	5.5	0.34
	28S	4.3	0.4	0.97	5.3	0.34

RNA Quality Assessed By:

- 28S/18S Height Ratio
- 28S/18S Area Ratio
- 28S Fraction of Total Area
- 18S Fraction of Total Area
- Fast Area Ratio
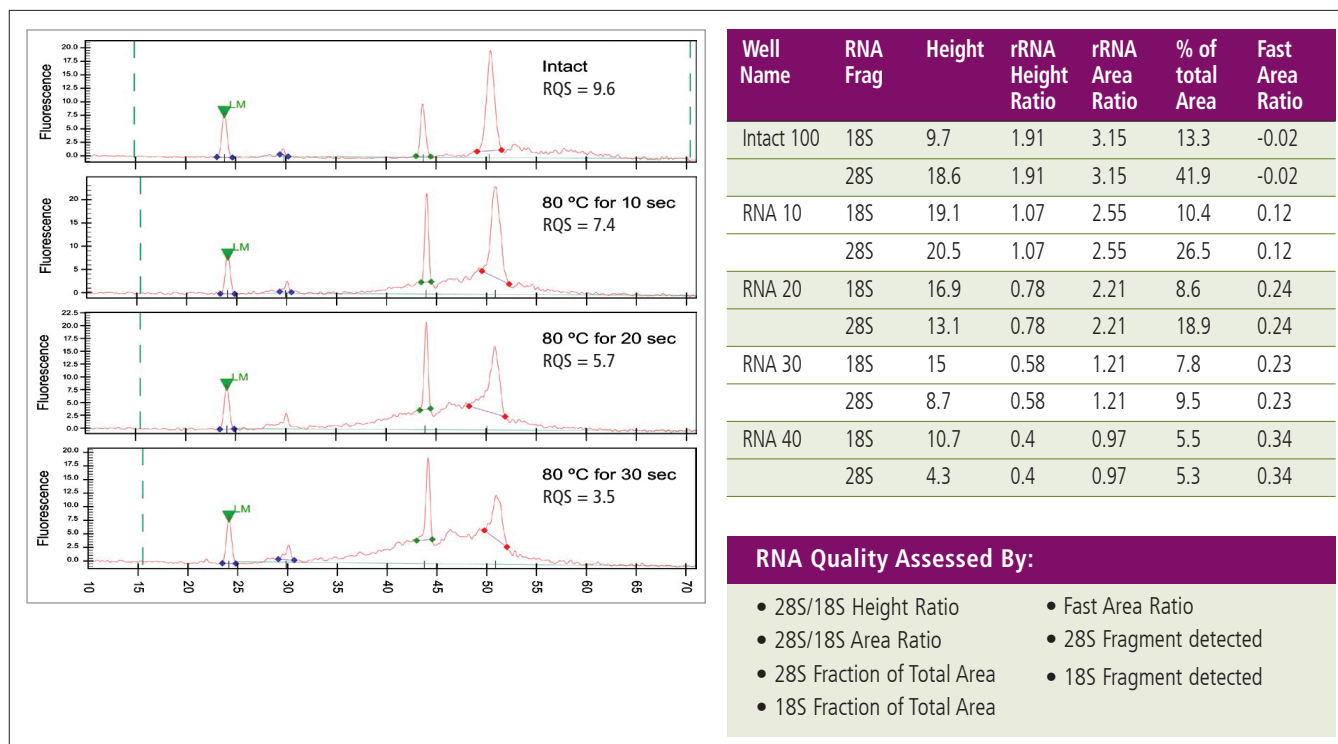- 28S Fragment detected
- 18S Fragment detected

Figure 3. (left) shows a progression of degradation of RNA sample, from top to bottom. The intact RNA was intentionally degraded by heating over a prescribed period of time illustrating sample degradation. An individual ratio or a combination of ratios can be used as appropriate to assess the quality of the RNA samples (Figure 3, right).

Using the DV$_{200}$ metric as a Quality Score

The DV$_{200}$ is a second quality metric available with the RNA assay. This metric is promoted by Illumina to improve on the quality assessment of FFPE samples where the RQS may not be as sensitive a measure. The DV$_{200}$ calculates the percentage of RNA fragments which are greater than 200 nucleotides. FFPE can cause significant RNA degradation in some samples. Thus, it is very important to access RNA quality after FFPE extraction of RNA for downstream applications. Samples do not progress to downstream processes after a hard cutoff of 30. Data shown in Figure 4 is courtesy of Broad Institute Genomics Platform.
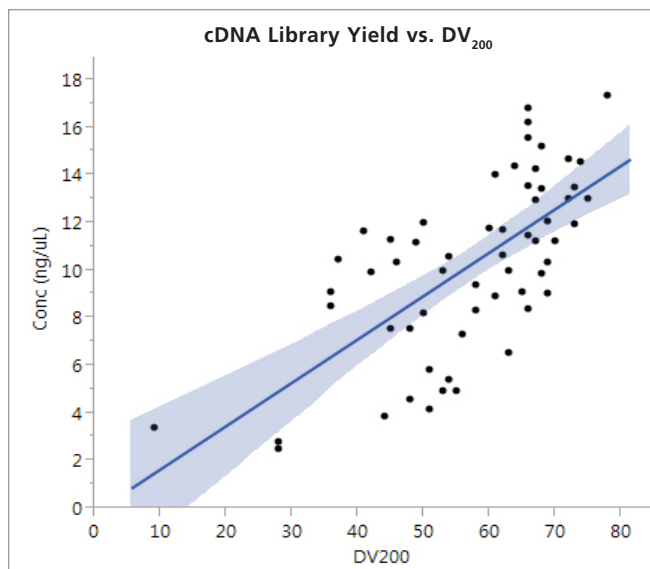

Figure 4. The DV$_{200}$ measure correlates well with cDNA library quantification and is can be used to assess likelihood of success

LabChip GX Touch RNA Analysis	
Linear Range:	25 to 250 ng/µl (high range)
	5 to 50 ng/µl (low range)
	500—5000 pg/µl (with Pico RNA Reagent Set)
Quantification Reproducibility:	<20% CV (from chip to chip and instrument to instrument)
Quantification Accuracy:	<+/- 30% error with ladder as sample
Size Range:	100 to 6000 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 9 (about 2.5 hours for 96-well plate)
Setup Time:	About 1/2 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 —> 1000 samples per reagent kit.
Chip Lifetime	>2000 samples

Ordering Information	LabChip GX	LabChip GX Touch HT	LabChip GX Touch 24
DNA/5K/RNA/CZE LabChip	760435	760435	CLS 138949
Standard RNA Reagent Kit	CLS 960010	CLS 960010	CLS 960010
Pico RNA Reagent Kit	CLS 960012	CLS 960012	CLS 960012