

## The ScreenTape® Degradation Value (SDV), a new standard for RNA QC

Lab901's ScreenTape Degradation Value (SDV) is an RNA QC metric that is specifically optimised for and fully integrated with Lab901's ScreenTape R6K platform. In an independent benchmarking study, SDV has been shown to be equivalent to the RIN for total RNA quality assessment and to offer better discrimination for poorer quality samples. Lab901's software calculates RNA degradation based on factors that avoid the misinterpretation of RNA electropherograms. Generation of the SDV is independent of ribosomal peak volumes and therefore avoids the inherent uncertainty of relying on peak boundaries. The result is an RNA QC metric that can reliably and reproducibly help decide which samples can be used for microarray and qRT-PCR experiments.

### Introduction

The SDV is a novel RNA quality metric that has been specifically tailored to Lab901's ScreenTape R6K platform. The SDV has been benchmarked against Agilent's RNA Integrity Number (RIN) by LGC, the leading UK National Standards Agency, and was shown to be comparable in terms of reproducibility and discrimination between different levels of RNA degradation. In this study the SDV was found to be a robust measure for accurate and reproducible RNA quality assessment. According to LGC it therefore qualifies as an alternative metric for RNA sample quality control, and a useful predictor of downstream microarray performance. This technical note describes the properties of the SDV, presents the results from LGC's benchmarking study and presents an SDV to RIN conversion chart.

### How does the SDV work?

The SDV is derived from a mathematical model that calculates an objective quantitative measurement of RNA degradation. When separated by electrophoresis, eukaryotic total RNA samples display three major peaks representing small RNAs, 18S ribosomal RNA (rRNA) and 28S rRNA. However, underlying these dominant profile features are low levels of a complex population of RNAs that cover a wide spectrum of molecular weights. As total RNA degrades, the 28S and 18S peaks slowly disappear while peaks from degraded material emerge in the region between the 18S and small RNAs. The SDV represents the ratio of the average degradation peak signal to the 18S peak signal multiplied by 100. In short, the higher the SDV, the higher the level of RNA degradation. The SDV is independent of rRNA peak selection boundaries and unaffected by sample concentration. It can therefore facilitate eukaryotic total RNA QC analysis in a more objective and reproducible manner than many other mainstream RNA QC methods.

### Samples used for SDV to RIN benchmarking study (by LGC)

Total RNA was extracted using TRIzol® LS Reagent (Invitrogen) from HepG2 cells that were untreated or exposed media supplemented with 0.5% (v/v) DMSO vehicle (Sigma Aldrich) and 4 mM ACAP (Paracetamol, Sigma Aldrich). RNA quantity was determined using a NanoDrop 1000 spectrophotometer. RNA at 1µg/µl from the HepG2 cells was then heated at 90°C for 0 to 21 minutes. After single colour labelling, these RNA samples were hybridized to Agilent Homo sapiens 4 × 44K whole genome gene expression arrays according to the manufacturer's instructions and scanned on an Agilent G2505B scanner. The resulting expression data was analysed using GeneSpring. For benchmarking RNA integrity (RNA samples at 25ng/µl), the TapeStation® was used in conjunction with ScreenTape R6K, and compared to the 2100 BioAnalyzer (Agilent Technologies) with the RNA 6000 series II Nano LabChip analysis kit.

### Example of results obtained on ScreenTape

Data for the RNA samples is displayed as a gel image as well as an electropherogram. Figure 1 shows the results for RNA samples of different qualities that have been run and analysed in quadruplicate on the ScreenTape R6K system. Each sample run on the system can either be viewed as an electropherogram or in a familiar gel image. SDV values are automatically

generated by the software on completion of the electrophoresis and can be exported with a single mouse click to Excel, Word or a PDF report. Figure 2 displays how electropherograms from different samples can be overlaid for comparison.

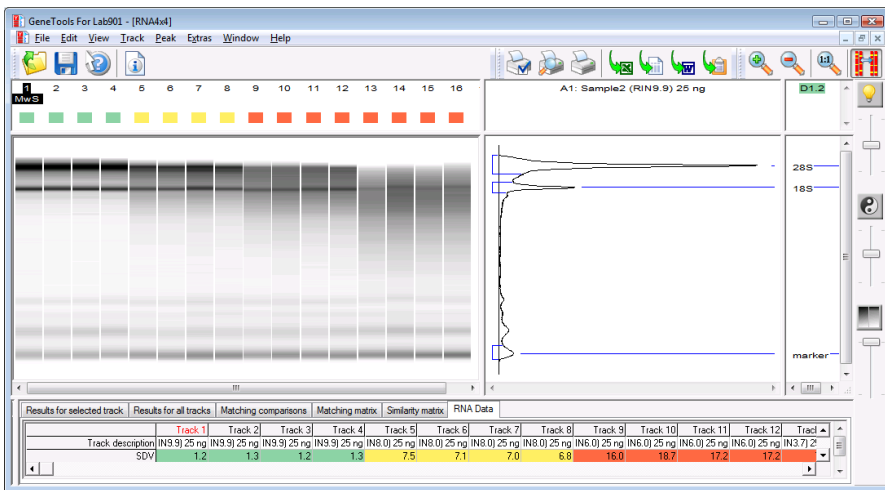


Figure 1: Analysis of total RNA extracted from HepG2 cells (eukaryotic) on ScreenTape R6K. Lanes 1 to 16 contain different RNA preparations that are either good quality (no heat degradation) with SDVs of 1.2, 1.3, 1.2 and 1.3 in lanes 1 to 4; medium quality (heat degraded for 6 minutes) with SDVs of 7.5, 7.1, 7.0 and 6.8 in lanes 5 to 8, poor quality RNA (heat degraded for 12 minutes) with SDVs of 16.0, 18.7, 17.2 and 17.2 in lanes 9 to 12 and very poor quality RNA (heat degraded for 21 minutes) with SDVs of 73.2, 66.0, 67.5 and 70.1 in lanes 13 to 16. All samples contain a bottom marker of 50b that is used as an inlane standard. Results for the SDV are colour coded (green, amber and red) corresponding to the SDV returned and allowing for rapid RNA QC scoring. Conveniently, these colour coding boundaries can be tailored to your own applications at any time.

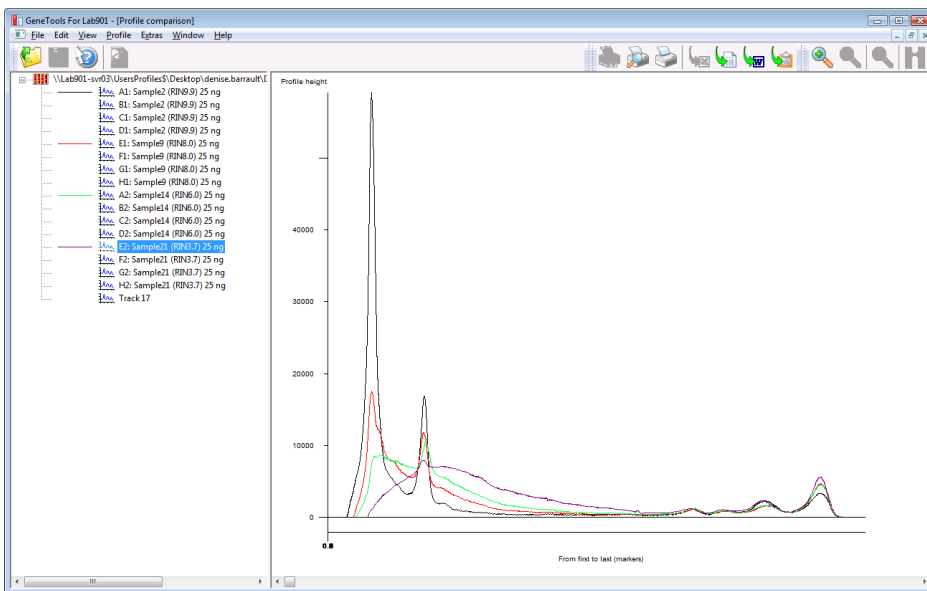


Figure 2: Overlay of sample profiles from lanes 1, 5, 9 and 13 of Figure 1. This sample comparison clearly shows the gradual disintegration of the 28S and 18S peaks into smaller RNA fragments that have greater mobility during electrophoresis.

As shown in Figure 3, when total RNA samples with known levels of degradation are run at different concentrations, the SDV values returned by the TapeStation remain unchanged, showing that the platform is robust to variations in RNA concentration and therefore that the SDV is not concentration dependent.

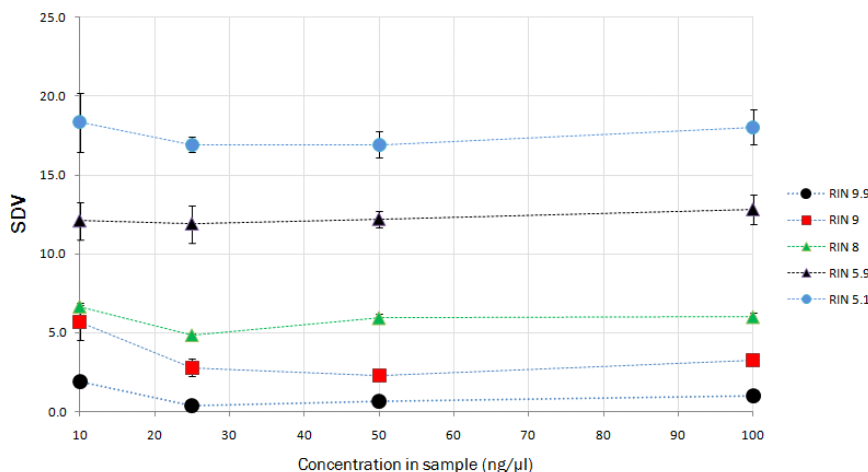


Figure 3: The graph presented illustrates that the SDV is robust and not concentration dependent. Samples of known levels of degradation were run at different concentrations on the TapeStation. Results show that there is no significant difference in the SDV values across the studied concentration range.

### Comparison of TapeStation and BioAnalyzer results

As shown in Figure 4, both SDV and RIN are suitable quality measures for classifying the panel of heat degraded total RNA samples extracted from the HepG2 cells. The data also highlights the increased uncertainty over RNA quality classification when samples are more degraded (SDV value  $\geq 15$  or RIN value  $\leq 6$ ). In this context the SDV correlates well with the RIN but offers the advantage of a better classification performance. For microarray data, rank correlations with treatment and intra-class correlations are high between the SDV and the RIN. Classification methods show that the majority of observations were classified into appropriate treatment groups by both RIN and SDV, with a slightly better classification performance of the SDV metric, which was significant at the  $P \leq 0.05$  level for two out of the four classification methods used. This provides significant benefits for laboratories processing poorer quality total RNA samples, such as those extracted from FFPE samples, biopsies or laser micro dissected tissue.

The SDV therefore offers an alternative to currently available metrics for RNA integrity analysis and provides a performance comparable to that of the RIN. The rapid assay time, compelling ease of use and flexible low, medium and high throughput capacity of the TapeStation means it is ideal for laboratories that process large or small numbers of total RNA samples.

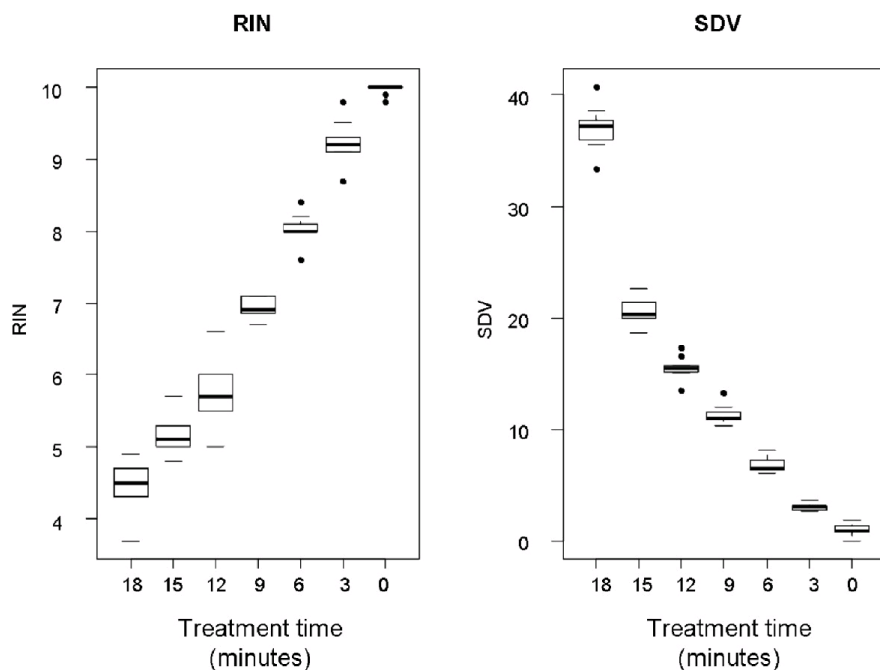


Figure 4: Relationship between SDV and RIN using heat degraded samples. Comparison of the SDV and RIN integrity metrics for a panel of seven RNA samples. "Treatment" denotes the duration of thermal degradation treatment used. Each group includes nine observations, except for treatment four which corresponds to a 12 minute incubation at 90 °C, which includes only seven observations. The thicker horizontal line shows the median, boxes show the upper and lower quartiles and whiskers extend to the most distant data point within 1.5 times the interquartile range of the relevant quartile. Values beyond this are shown as individual data points. Data copied and reproduced with permission from Wilkes *et al.* (2010).

### Correlation and relationship between the SDV and RIN

Using the RNA QC data collated by the LGC benchmarking study, we mapped the relationship between the SDV and RIN. Figure 4 shows the results from LGC's study plotting SDV values against RIN values. This reveals an exponential relationship between the two RNA quality metrics. Using the equation from the best fit exponential trend line ( $R^2=0.96$ ) it is possible to generate a conversion table for easy SDV to RIN comparison, which is displayed in Table 1.

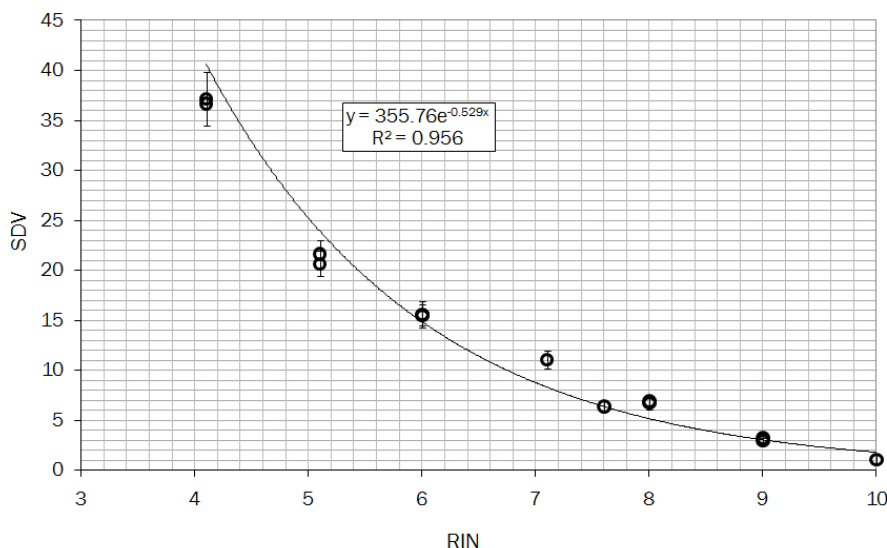


Figure 4: Graph showing corresponding RIN and SDV values for the samples used in the LGC benchmarking study. The trend line in this graph shows the exponential relationship between the two values with an  $R^2$  of 0.956.

Very Good RNA		Good RNA		Medium RNA	
SDV	RIN	SDV	RIN	SDV	RIN
1	9.90	6	7.72	10	6.75
2	9.79	7	7.43	12	6.41
3	9.03	8	7.17	14	6.12
4	8.48	9	6.95	16	5.86
5	8.06	10	6.75	20	5.44

Poor RNA		Very poor RNA		Extremely poor RNA	
SDV	RIN	SDV	RIN	SDV	RIN
25	5.02	110	2.22	160	1.51
50	3.71	125	1.98	200	1.09
75	2.94	135	1.83	230	0.82
85	2.71	140	1.76	250	0.67
100	2.40	150	1.63	300	0.32

Table 1: Conversion chart from SDV to RIN.

### Benefits of using SDV for eukaryotic RNA QC

- The SDV has been shown by LGC to be a robust RNA quality metric that compares with the RIN in terms of reproducibility and discrimination between different levels of RNA degradation, therefore giving you confidence in your RNA QC results.
- The SDV correlates well with the RIN but offers the advantage of a better classification performance for more degraded samples, traditionally the case for RNA extracted from FFPE samples, biopsies or laser micro dissected samples.
- The SDV is fully integrated to the ScreenTape R6K platform, providing a fully automated and walk-away solution for RNA QC without any chip priming or reagent preparation.
- SDV results are colour coded for convenience during sample scoring. It is possible to modify the boundaries of the colour coding at any time to suit your experiment or application.
- The SDV allows you to compare different RNA samples regardless of their origin and concentration so that you can compare their quality between labs, between experiments and after storage.

### Reference

Wilkes T. M. *et al.* (2010) Evaluation of a novel approach for the measurement of RNA quality. *BMC Research Notes* **3:89**.

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