

ScreenTape® R6K streamlines accurate and objective Quality Control (QC) of eukaryotic total RNA

Tape Used

ScreenTape R6K

Total time to result

One minute per sample

Sample volume per test

2µl

Qualitative range

0.5 to 100ng/µl

Limit of detection: 100pg/µl

Analysis Results

ScreenTape Degradation Value (SDV)

Automated 28S and 18S peak location and annotation, 28S/18S ratio

Custom RNA quality colour coding

Prior to qRT-PCR and microarray experiments, assessment of RNA integrity is achieved easily with the Lab901 ScreenTape R6K platform, in one minute per sample. ScreenTape R6K provides automated, hands-free RNA analysis without any reagent preparation or chip priming, allowing you to accelerate your RNA workflow. Fully integrated software returns the ScreenTape Degradation Value (SDV): an objective quality score for mammalian total RNA, which measures and classifies RNA degradation. Through automation and process control, R6K will increase your confidence in RNA Quality Control (QC) procedures, and eliminate your RNA analysis bottleneck.

Introduction

Total RNA isolated and purified from *in vitro* cell cultures, tissue biopsies or clinical samples is the starting material for many applications such as qRT-PCR, microarray analysis and Northern blotting. After RNA purification, laboratories routinely analyse RNA integrity using techniques such as gel electrophoresis or capillary electrophoresis. Up until now these techniques have required labour intensive reagent preparation or chip priming, lengthy run times, and in many cases complex and subjective analysis. The Lab901 ScreenTape R6K system is a novel, automated method for total RNA analysis that provides a simple hands-off solution for RNA QC.

ScreenTape RNA analysis procedure

Good quality total RNA was purified from HepG2 cells. Aliquots of the isolated material were degraded for 0 to 21 minutes at 90°C to produce RNA of seven different qualities. The resulting material was mixed in equal volumes with R6K loading buffer and heated to 72°C for 3 minutes, ready for analysis on R6K ScreenTape. A Norgen Biotek RNA ladder was prepared for R6K analysis in the same manner. After preparation, all samples were transferred in their tubes to the TapeStation®, where ScreenTape R6K and tips were also placed. After clicking “START” on the software driven menu, full analysis of the samples was achieved and archived, with no user intervention, within one minute per sample.

Results on ScreenTape R6K

Typical Lab901-Genetools™ screen grabs, showing results for the different HepG2 RNA samples, are displayed in Figures 1 to 3. In Figure 1, the electropherogram for lane 4 shows that 28S and 18S ribosomal sub-unit peaks have been automatically identified and annotated by the integrated software. This is a good quality RNA sample, with an SDV of 5.6, where these two peaks are distinct and only a few degraded products are visible downstream of the 18S peak in the gel image and electropherogram profile. Small RNAs are also clearly visible in the lower half of the electropherogram just upstream of the bottom marker, which is a standard component of the R6K loading buffer. As the quality of the RNA in lanes 2 to 8 decreases, degradation products accumulate downstream of the 18S peak, eventually resulting in the ribosomal subunit peaks becoming less and less defined. A major feature of R6K is the automated delivery of the ScreenTape Degradation Value (SDV), an objective RNA quality value that measures the level of eukaryotic total RNA degradation. The higher the SDV the more the RNA sample is degraded. The SDV, which is a customised version of the Degradometer: an RNA quality metric developed by Auer *et al.* (2003)ⁱ, helps you assess the quality of your RNA sample impartially. An independent, peer reviewed study carried out by Wilkes *et al.* (2010) at LGC, the UK's National Measurement Institute for chemical and bioanalysis, qualified the SDV as a robust metric for RNA sample quality control, and a useful predictor of downstream microarray performanceⁱⁱ. SDV is also an acceptable metric for RNA QC prior to RT-qPCR according to MIQEⁱⁱⁱ guidelines^{iv}.

Results in Figure 1 show that the SDV increases from 0.9 to 67.2 in lanes 2 to 8, reflecting the increasing levels of RNA degradation across the tape. SDV results are colour coded into three categories (green, yellow, red) to allow rapid visual assessment of RNA quality. You can define the boundary SDVs for the colour coding to suit your downstream application.

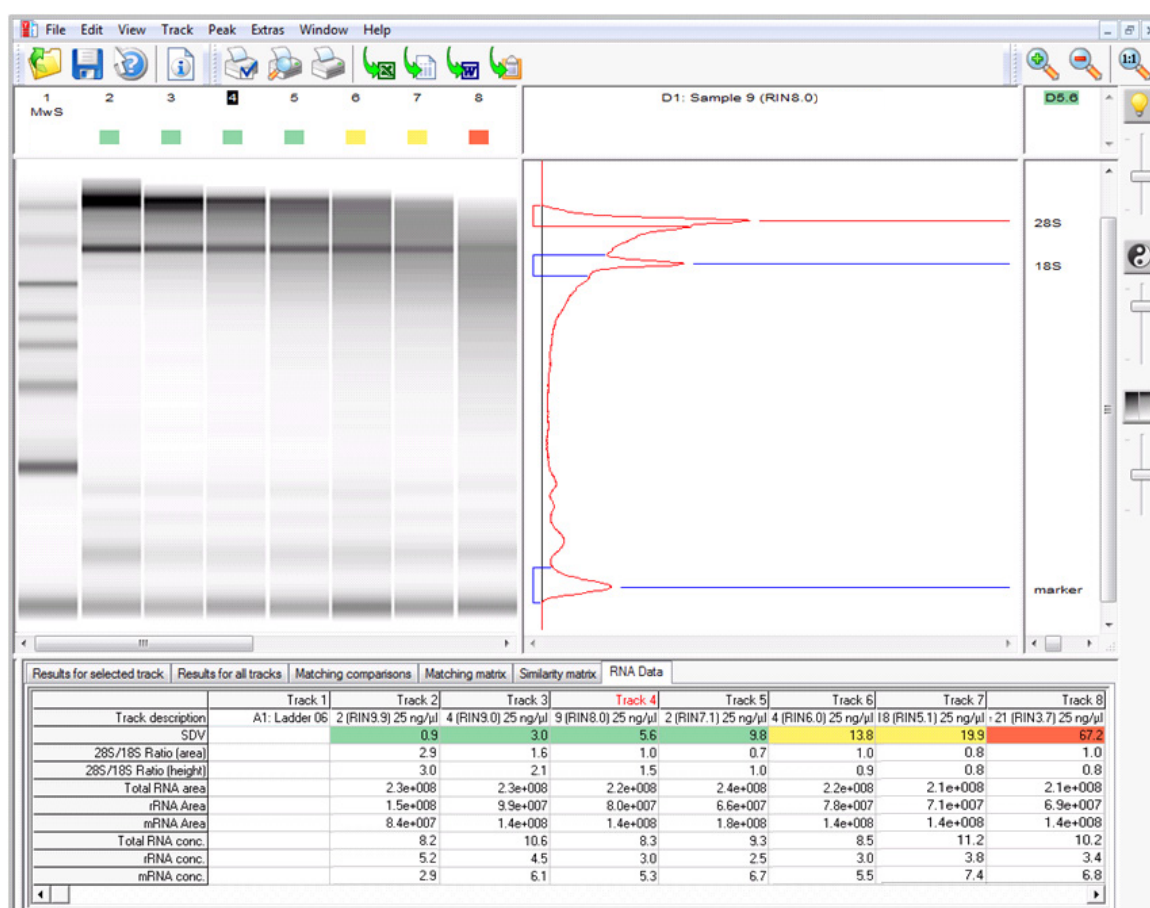


Figure 1: Quality assessment of HepG2 cell total RNA on ScreenTape R6K. Lane 1 contains an RNA ladder, showing discrete bands at 4kb, 2kb, 1.2kb, 800b, 600b, 400b and 200b. All lanes contain an in-lane bottom marker of 50b. Total RNA extracted from HepG2 cells at 25ng/µl was heat-degraded at 90°C for 0 to 90 minutes, resulting in seven total RNA samples with increasing levels of degradation that were run in lanes 2 to 8. Results show a gel image for each sample and an annotated electropherogram highlighting 28S, 18S and the marker peak. Small RNAs are visible as three small peaks upstream of the bottom marker. The results table contains all SDV values for the analysed samples as well as 28S/18S peak ratios and other values such as total RNA and mRNA area volumes. The SDVs increase from 0.9 to 67.2 in lanes 2 to 8. Samples are colour flagged (green for good, amber for average or red for bad) according to SDV boundaries you can define. In this example the boundaries were set at 10 and 20 SDV units.

In Figure 2, 16 replicate RNA samples that were heat degraded for 3 minutes were run across a whole ScreenTape, showing the reproducibility of the R6K platform. In a similar experiment mean SDVs for RNA samples degraded for 0, 3, 9 and 21 minutes (samples 1 to 4 respectively) showed CVs below 5%, demonstrating that SDV is a precise quality metric for total RNA (data shown in Figure 3). The integrated Lab901-GeneTools analysis software also calculates and displays more traditional RNA quality metrics such as 28S to 18S peak area ratios, which are presented in the tabulated results in Figures 1 and 2.

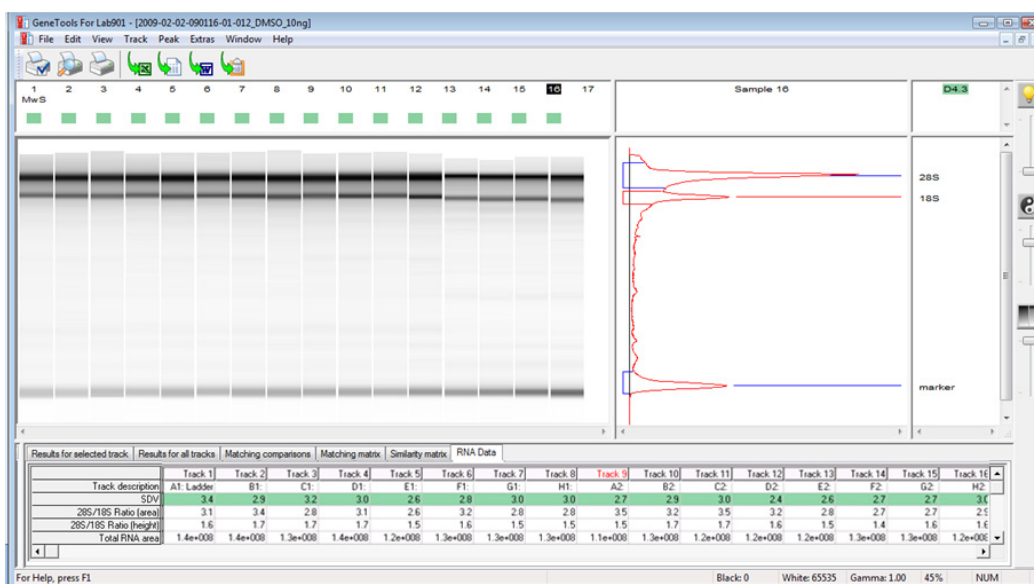


Figure 2: ScreenTape R6K is a reproducible method for RNA QC. 16 replicates of RNA degraded for 3 minutes at 90°C were run on ScreenTape R6K and showed a mean SDV of 2.9 with a CV < 10%.

The combination of SDV, peak profiles and ribosomal peak metrics will provide you with enough data to objectively determine RNA degradation in your test samples and will enable rigorous and impartial RNA QC. All the data generated by the ScreenTape platform is automatically archived in a GLP compliant format, allowing your RNA QC records to be up to date and easily accessed. Results can also be exported to Excel or Word in a single mouse click, for further analysis or easy report writing.

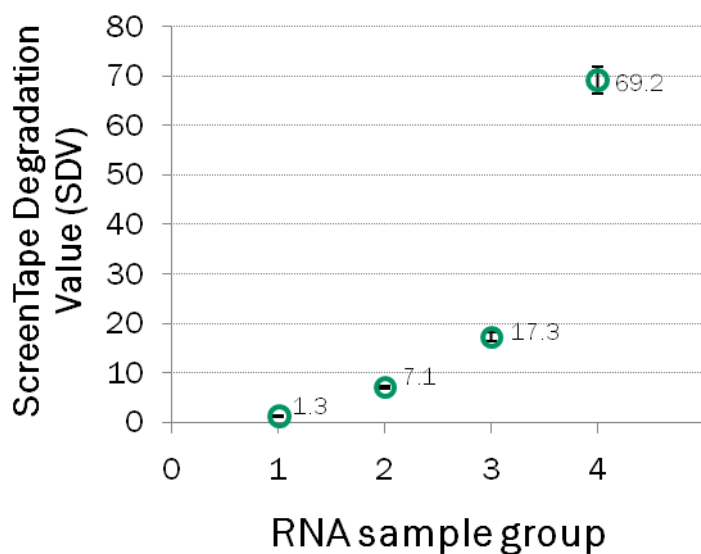


Figure 3: Graph showing the standard deviations on SDV values returned for 4 replicates of RNA samples labeled 1 to 4 that were degraded for 0, 3, 9 and 21 minutes and run on the same ScreenTape. Overall SDV values for replicate samples have a CV < 5%.

Benefits of using ScreenTape R6K for total RNA QC.

The ScreenTape R6K system will simplify objective eukaryotic total RNA quality assessment, improving your laboratory workflow for RNA related applications:

- **Convenient** - ScreenTape R6K is pre-packaged, which eliminates all reagent preparation and chip priming. This results in a convenient and highly reproducible RNA analysis method.
- **Cost effective** - The discreet sample channels on ScreenTape R6K allow you to consume unused lanes at a later date. There is no need to batch process QC samples, which gives you greater workflow flexibility.
- **Fast** - At a sample per minute, ScreenTape R6K will accelerate your RNA QC processes.
- **Compact and integrated** - The compact TapeStation combines sample loading, RNA separation, profile imaging and sample analysis, which saves you valuable laboratory space and reduces your manual labour.
- **Objective RNA QC** - The SDV, an objective measurement of RNA degradation, provides straightforward and precise evaluation of eukaryotic total RNA quality. This means that you can easily compare different RNA batches and remove all subjectivity from RNA QC analysis.
- **Familiar** - RNA integrity and characterisation can also be assessed using more familiar methods such as the gel image, RNA separation profile with annotated 28S and 18S ribosomal peaks or ribosomal peak metrics.
- **Compliant** - Results are seamlessly saved and archived in a format that is GLP compliant, so that your laboratory records are precise and up to date at all times.

To discover more RNA applications that can benefit from the ScreenTape platform, please visit www.lab901.com.

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ⁱ Auer, H., Lyianarachchi, S., Newsom, D., Klisovic, M.I., Marcucci, G., and Kornacker, K (2003) Chipping away at the chip bias: RNA degradation in microarray analysis. *Nature Genetics*, **35** (4), 292-3.

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

ⁱⁱⁱ MIQE: Minimum standard for the provision of Information for qPCR Experiments.

^{iv} Bustin S.A. (2010) Why the need for qPCR publication guidelines? – The case for MIQE. *Methods*, **50**, 217-226.