Quality Control for SureSelect Strand-Specific RNA Library Preparation Using the Agilent 2200 TapeStation System

Application Note

Nucleic Acid Analysis

Authors

Arunkumar Padmanaban
Agilent Technologies, Inc.
Bangalore, India

Alex Siebold
Agilent Technologies, Inc.
Santa Clara, CA
USA

Abstract

This Application Note details a reliable and reproducible method for performing the recommended quality control (QC) steps of total RNA starting materials and corresponding cDNA libraries synthesized for RNA Sequencing (RNA-Seq) applications using the Agilent 2200 TapeStation system. Starting total RNA and final cDNA libraries generated with the Agilent Strand Specific RNA library preparation workflow were analyzed for sample integrity and quantity on the 2200 TapeStation system, using the RNA ScreenTape and D1000 ScreenTape assays. Results were then compared to the Agilent 2100 Bioanalyzer system, using the RNA 6000 Nano and DNA 1000 assays. Analysis of the samples tested indicate that the performance of the RNA and D1000 ScreenTape assays for the 2200 TapeStation system are equivalent to the existing RNA 6000 Nano and DNA 1000 assays run on the 2100 Bioanalyzer system. These findings show that the 2200 TapeStation system, when partnered with the RNA or D1000 ScreenTape assays, serve as an easy-to-use application for sample QC within the RNA-Seq workflow, offering flexible sample throughput.

Agilent Technologies

Introduction

The Agilent SureSelect strand-specific RNA library preparation kit generates libraries with specific adaptors ligated to each strand, enabling the identity of the DNA template strand of origin to be retained in downstream sequencing and data analysis. As high-throughput sequencers continue to generate more data for a single experiment, compared to other methodologies such as RT-PCR and microarrays, large scale RNA-seq analysis often involves significant computing and monetary resources. Therefore, to perform RNA-seq based experiments, accurate and reliable quality control measurements of the initial starting materials and the samples as they progress through the library preparation workflow are a crucial step to ensure the best quality data are generated after sequencing and informatics analysis is completed.

The Agilent 2200 TapeStation system offers an easy-to-use automated electrophoresis system with rapid analysis time of 1 to 2 minutes per sample as well as offering flexible throughput capabilities compared to the Agilent 2100 Bioanalyzer system. This Application Note describes and compares the performance and capabilities of the RNA and D1000 ScreenTape assays for use on the 2200 TapeStation system with comparable RNA and DNA assays run on the 2100 Bioanalyzer system. The results from the integrity control of the starting total RNA and final sequencing libraries generated from an Agilent SureSelect strand-specific RNA library preparation kit reveal that the D1000 ScreenTape, as well as the RNA ScreenTape assay with its RNA integrity number equivalent (RIN^e), are a match to the RNA integrity number (RIN) and quantitative results obtained from the 2100 Bioanalyzer system, the latter of which is widely used for RNA QC, and has been used for the QC of next generation sequencing libraries.

Materials and Methods

Agilent 2200 TapeStation system (G2964AA), RNA ScreenTape and Sample Buffer (5067-5576 and 5067-5577), D1000 ScreenTape and reagents (5067-5582 and 5067-5583), Agilent 2100 Bioanalyzer system (G2939AA), DNA 1000 kit (5067-1504), and RNA 6000 Nano kit (5067-1511), Agilent SureSelect Strand Specific RNA Reagent kit (G9691A) for Illumina platform were obtained from Agilent Technologies. HEK293 cell lines were cultured as recommended[1] and total RNA was extracted using the Agilent Absolutely RNA Miniprep Kit (400800). Total RNA QC was carried out using the RNA ScreenTape assay and RNA 6000 Nano kit on the 2200 TapeStation and 2100 Bioanalyzer systems respectively. Strand-specific RNA-seq library synthesis was carried out following the SureSelect Strand-Specific RNA Library Prep for Illumina Multiplexed Sequencing – mRNA Library Preparation Protocol (version A.3)[2]. QC of the synthesized cDNA libraries was carried out using the D1000 ScreenTape assay with the 2200 TapeStation system. The performance was compared to the same samples run on the 2100 Bioanalyzer system using the DNA 1000 kit. As per the SureSelect protocol, cDNA library sizes were measured from the peak maxima on the 2200 TapeStation or 2100 Bioanalyzer systems. Quantity determination of cDNA library samples was measured using the region functionality. All samples were run in replicates of six on each system. Unless stated, the manufacturer's protocols and guidelines were followed.

Results and Discussion

Figure 1 illustrates the SureSelect protocol for the generation of strand-specific RNA-Seq libraries using total RNA as the starting material. The first step of the protocol consists of the poly(A) selection of the total RNA using oligo(dT) coupled magnetic beads. In brief, following poly(A) selection, the samples undergo fragmentation, first strand cDNA synthesis, second strand cDNA synthesis, end-repair, A-tailing, adapter ligation, and PCR amplification, during which the adapter ligated fragments are selected, amplified, and indexes (that is, barcodes) are incorporated to uniquely tag each sample being processed. Following PCR, the amplified adaptor ligated libraries are cleaned up using SPRI beads then analyzed to ensure the proper size, quantity, and purity (that is, absence of adaptor-dimer products) of the libraries are obtained before sequencing.

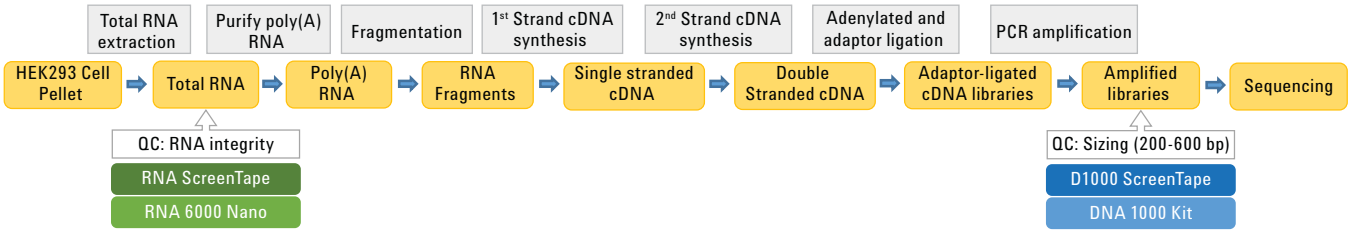

Figure 1. Detailed workflow showing the QC steps involved and the associated assays of the Agilent 2200 TapeStation system (dark green and dark blue) and the Agilent 2100 Bioanalyzer system (light green and light blue).

Total RNA quality control

Quality control of total RNA to determine its integrity is essential to ensure that high quality starting material is used for the library preparation. The total RNA isolated from the HEK293 cell lines was analyzed using the RNA ScreenTape and RNA 6000 Nano assays on the 2200 TapeStation and 2100 Bioanalyzer systems, respectively. The SureSelect protocol indicates that a RNA integrity number of 8 or above should be used for RNA library preparation. Gel-like images generated by the analysis software from both instruments are presented in Figure 2 together with calculated RIN^e and RIN values with an average of 9.5.

Table 1 summarizes the RNA integrity and quantity metrics obtained from both instruments, and shows that the 2200 TapeStation system is highly comparable to the 2100 Bioanalyzer system for quantifying and analyzing the integrity of total RNA.

Library quality control

Following the integrity analysis on the total RNA, the poly(A) RNA was then purified from the total RNA and further processed following the SureSelect protocol to synthesize the final cDNA sequencing libraries. The synthesized libraries were then analyzed using the D1000 ScreenTape assay on the 2200 TapeStation system and the DNA 1000 kit on the 2100 Bioanalyzer system.

This QC step ensures that the libraries are of proper size, free from primer dimers that may be introduced during PCR, and that sufficient yield of each library are obtained for sequencing. Figure 3 and Figure 4 respectively show the gel-like images of the final cDNA libraries assayed, as well as their corresponding electropherograms.

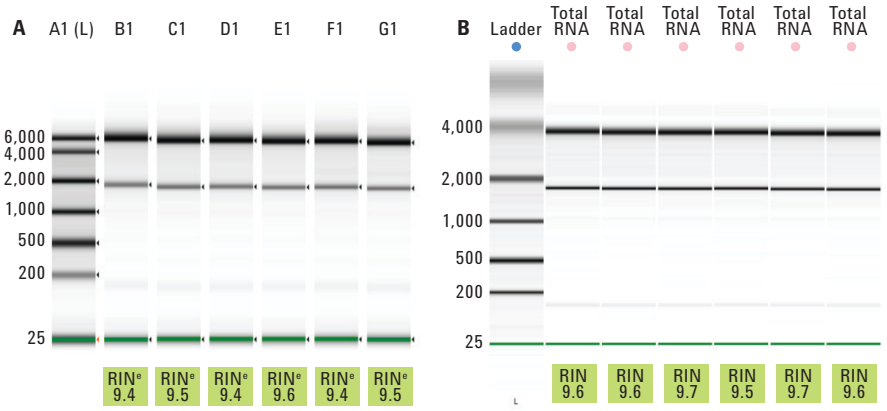

Figure 2. A) Gel-like image of total RNA run on the Agilent 2200 TapeStation system. B) Gel-like image of the total RNA run on the Agilent 2100 Bioanalyzer system.

Table 1. Quantitation and integrity assessment of total RNA (n = 6) as measured by the Agilent 2200 TapeStation and the Agilent 2100 Bioanalyzer systems.

	Quantitation (ng/µL)		Integrity (RIN^e/RIN)	
System	Agilent 2200 TapeStation	Agilent 2100 Bioanalyzer	Agilent 2200 TapeStation	Agilent 2100 Bioanalyzer
Average	128.8	116.5	9.5	9.6
CV %	2.8	7.4	0.9	0.8

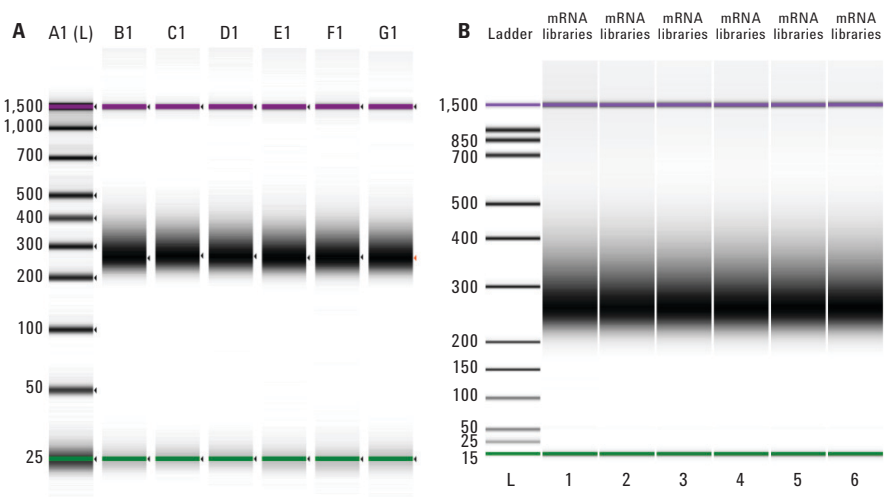

Figure 3. A) Gel image of cDNA library run on the Agilent 2200 TapeStation system. B) Gel-like image of the cDNA library run on the Agilent 2100 Bioanalyzer system.

Using the region functionality on the 2200 TapeStation and 2100 Bioanalzyer systems, the libraries are distributed between 170–600 bp, with a peak maxima of 266 bp and 263 bp respectively. This is within the recommendations of the target enrichment protocol. The absence of any peaks in the 100–150 bp region in the electropherograms clearly shows that the libraries are free of any adaptor-dimer products. Similar to the results displayed in Table 1. Table 2 also demonstrates that the quantity and sizing metrics obtained from both instruments, following the analysis of the final cDNA sequencing libraries, are highly similar, providing strong evidence that the 2200 TapeStation system is equivalent to the 2100 Bioanalyzer system for the QC of both total RNA and cDNA sequencing libraries generated with an Agilent SureSelect strand-specific RNA library preparation kit.

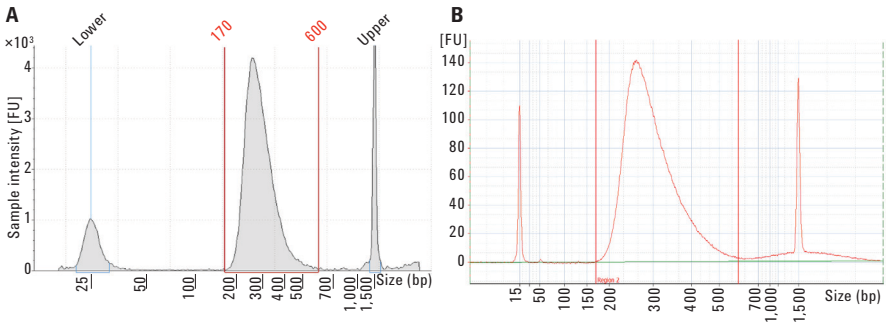

Figure 4. Electropherograms of cDNA library from the Agilent 2200 TapeStation system (A) and the Agilent 2100 Bioanalyzer system (B) showing the library size ranging from 170–600 bp.

Table 2. Quantitation and sizing data of the final cDNA sequencing libraries (n = 6) as measured by the Agilent 2200 TapeStation and the Agilent 2100 Bioanalyzer systems.

	Quantitation (ng/µL) (170–600 bp)		Peak maxima size (bp)	
Systems	Agilent 2200 TapeStation	Agilent 2100 Bioanalyzer	Agilent 2200 TapeStation	Agilent 2100 Bioanalyzer
Average	45.5	48.1	266	263
CV %	2.6	6.1	1.3	0.7

Conclusion

The results presented in this Application Note demonstrate that the Agilent 2200 TapeStation system in combination with the RNA ScreenTape and Agilent D1000 ScreenTape assays is an ideal instrument for the analysis of total RNA starting materials and final cDNA libraries synthesized in RNA-Seq library prep workflows. In addition to its equivalent performance to the Agilent 2100 Bioanalyzer system, the 2200 TapeStation system enables a more flexible and scalable sample throughput and accelerates the RNA sequencing workflow.

References

1. Product Sheet 293 [HEK293] (ATCC® CRL1573™), HYPERLINK "http://www.atcc.org/~/ps/CRL-1573.ashx" www.atcc.org/~/ps/CRL-1573.ashx Accessed online: 02 Dec, 2013.

2. G9691-90010. SureSelect Strand-Specific RNA Library Prep for Illumina Multiplexed Sequencing. http://www.chem.agilent.com/library/usermanuals/Public/G9691-90010.pdf.

www.agilent.com/genomics/tapestation

Published in the USA, March 27, 2014
5991-4116EN

Agilent Technologies