Application Note

Genomics

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Quality Metrics for Nucleic Acids with the Agilent Fragment Analyzer and Femto Pulse Systems

Authors

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Abstract

Assessment of nucleic acid integrity is essential to the success of many downstream applications. The Agilent 5200 Fragment Analyzer system and the Agilent Femto Pulse system offer quick and easy user-friendly metrics for evaluating DNA, RNA, and genomic DNA quality and integrity. The versatility of these quality metrics allows them to be applied to various DNA and RNA samples from different organisms, extraction methods, and tissue types, including extremely degraded formalin-fixed, paraffin-embedded (FFPE) samples.

Introduction

To assess the quality and integrity of DNA and RNA, four quality metrics are used for the Agilent 5200 Fragment Analyzer system: DQN, GQN, RQN, and DV200. These metrics have been published in numerous articles and incorporated in protocols and literature by Illumina, PacBio, and Bionano Genomics.

Experimental

The experiments in this study were performed using a 5200 Fragment Analyzer system and can be replicated with comparable results on Agilent 5300 and 5400 Fragment Analyzer systems. An Agilent Femto Pulse system was also used, with an Agilent Genomic DNA 165 kb kit (p/n FP-1002-0275) and an Agilent HS RNA kit (15 nt) (p/n DNF-472).

Results and discussion

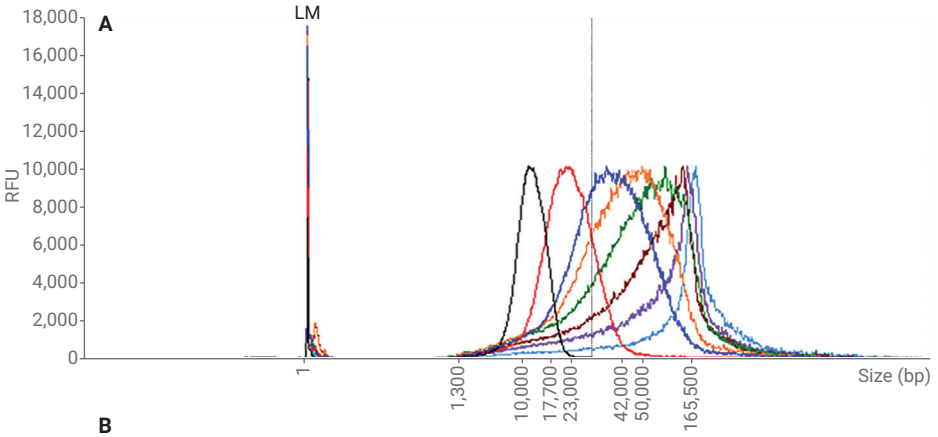
DQN and GQN

Agilent designed the DNA quality number (DQN) and the genomic quality number (GQN) for use in ProSize data analysis software to allow for easy analysis of sheared DNA and genomic DNA (gDNA) quality, respectively. The user defines a size threshold they deem appropriate for their specific application. ProSize then calculates a DQN or GQN value based on the fraction of the total measured concentration of the sample that lies above the specified size threshold. The DQN and GQN scores samples on a scale of 0 to 10. 0 indicates that none of the sample exceeds the threshold and 10 indicates 100 % of the sample lies above the threshold value. DNA size can vary due to numerous factors, such as: origin of organism, extraction methods, tissue type, and sample handling. Therefore, the ability to define the size threshold gives the user the advantage of defining quality DNA for their specific application.

GQN is used specifically for genomic DNA samples separated on the 5200 Fragment Analyzer system with the Agilent Genomic DNA 50 kb kit (p/n DNF-467-0500)and Agilent HS Genomic DNA 50 kb kit (p/n DNF-468-0500) and on the Femto Pulse system with the Agilent Genomic DNA 165 kb kit[1]. The DQN is used for all other DNA sample types and was created for samples that use a reagent kit with both a lower and upper marker.

As seen in Figure 1, sheared gDNA samples from PacBio of varying sizes were separated on the Femto Pulse system with the Agilent Genomic DNA 165 kb kit. The size threshold for these samples was set at 30,000 bp and reported a lower GQN for the smaller sized samples compared to the larger sized gDNA, as expected due to the varying size. The DQN and GQN values allow for fast and easy assessment of the quality of any type of DNA sample.

As a dynamic quality metric, the GQN brings objective analysis to the quality assessment of formalin-fixed, paraffin-embedded (FFPE) gDNA samples. FFPE DNA undergoes substantial chemical modifications during formalin fixation, including cross-linking and fragmentation. Utilizing the 5200 Fragment Analyzer system, Illumina developed three guidelines to follow based on the GQN value when preparing an FFPE library: (1) minimal DNA input into library preparation; (2) number of PCR cycles; and (3) the amount of library used in enrichment[2]. Successful and reliable library preparations and sequencing results can be achieved with the GQN metrics.



B

Average smear size (bp)	GQN Set at 30 kb
12,147	0
23,339	1.5
45,304	6.4
57,789	7.1
73,267	7.8
94,045	7.8
109,968	8.2
164,292	8.8

Figure 1. Sheared gDNA from PacBio separated on the Agilent Femto Pulse system with the Agilent Genomic DNA 165 kb kit. (A) Separations on the electropherogram (B) Average smear size, and $GQN_{30\,kb}$, n = 2, LM = lower marker.

RQN

ProSize uses the RNA quality number (RQN) as a quality metric for total RNA samples. ProSize considers the entire electropherogram including the 5S and fast region where the small RNA separates, as well as the small and large ribosomal peaks, the baseline resolution between the peaks, the ratio of the small and large ribosomal peaks, and the degradation in front of the small ribosomal peak when calculating the RQN. The 5200 Fragment Analyzer system can easily distinguish between small RNA fragments and degradation in the small RNA region, enabling ProSize to assign an accurate RQN. The RQN is based on a scale from 1 to 10 where 1 represents completely degraded total RNA and 10 represents intact total RNA. Universal mouse reference total RNA was separated on the 5200 Fragment Analyzer system with the Agilent HS RNA kit (15 nt) (Figure 2). Freshly prepared total RNA had a ribosomal ratio of 1.9 and an RQN of 9.7, while the same sample subjected to 70 °C for 10 minutes reported a ribosomal ratio of 0.1 and an RQN of 5.9. A large RQN value indicates higher quality RNA with minimal degradation in the sample. The concentration, RQN, and ribosomal ratio are automatically reported in ProSize, allowing for easy evaluation of total RNA quality.

Correlation work demonstrating that the RQN is equivalent to the RNA integrity number (RIN) from the Bioanalyzer was published in 2013[3]. Several peer reviewed publications have since stated that the RQN provided by the 5200 Fragment Analyzer system and the RIN provided by the Agilent 2100 Bioanalyzer system are equivalent indicators of RNA quality[4,5]. Both papers used the 5200 Fragment Analyzer system for assessing RNA quality and presented several electropherograms as examples. Application of the RQN can be found in the Application Note: *Plant RNA Degradation Detection with the Agilent 5200 Fragment Analyzer System*[6].

DV_{200} metric

FFPE RNA samples are difficult to use, as degradation due to fixation and storage conditions is often quite extensive. It is important to evaluate the quality of each FFPE RNA sample before proceeding with library preparation to eliminate highly degraded samples containing RNA fragments smaller than the optimal size range. Although RQN values are reliable metrics for evaluating the quality of RNA isolated from fresh tissue, it is not a definitive measure of RNA quality from FFPE samples. To solve this problem, Illumina used the Fragment Analyzer system to develop the DV_{200} metric[7], which calculates the percentage of RNA fragments greater than 200 nucleotides in size. The DV_{200} metric is used to determine the minimal RNA input required for successful library preparation and reproducible results. Given the strong correlation between DV_{200} values and library yield, the DV_{200} metric is ideal for assessing FFPE RNA quality prior to library construction[8].

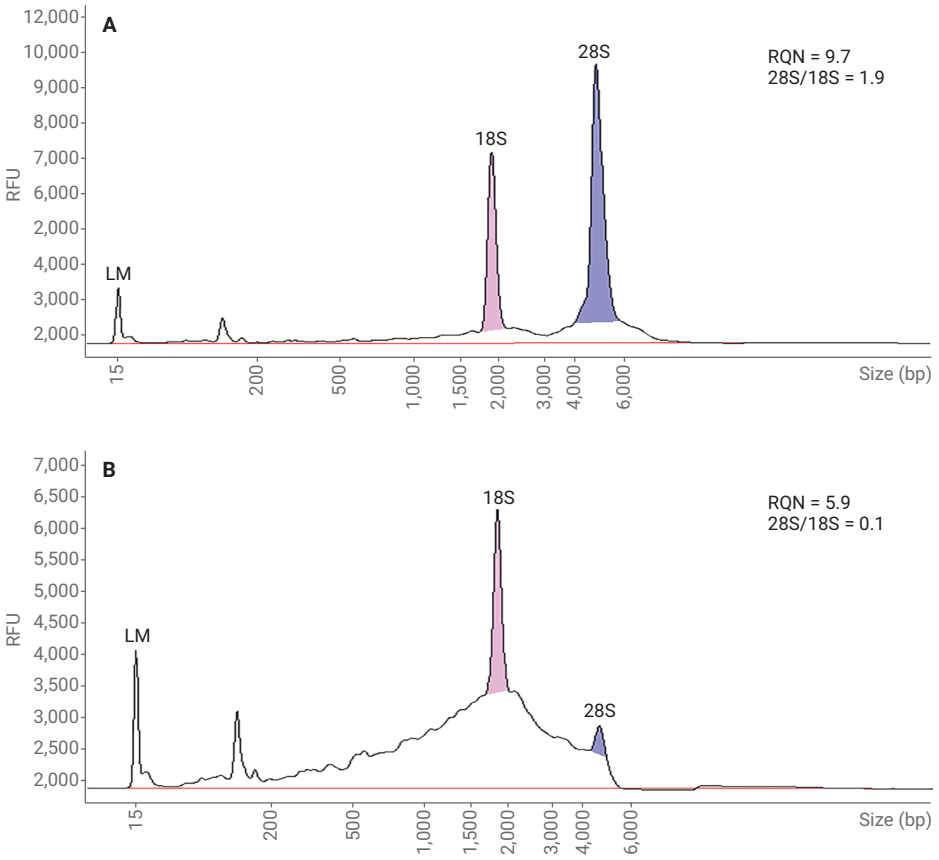


Figure 2. Universal mouse reference total RNA separated on the Agilent 5200 Fragment Analyzer system with the Agilent HS RNA kit (15 nt). (A) 0 minutes at 70 °C, RQN = 9.7. (B) 10 minutes at 70 °C, RQN = 5.9. LM = lower marker.

Conclusion

The DQN, GQN, RQN, and DV_{200} developed for the 5200 Fragment Analyzer system and Femto Pulse system provide reliable assessment of nucleic acid quality and integrity. The 5200 Fragment Analyzer system provides consistent quantification, sizing, and quality assessment for nucleic acids under 60 kb, while the Femto Pulse system is the only instrument capable of separating and sizing gDNA over 60 kb in as little as 70 minutes. The Femto Pulse system also has the added benefit of extreme sensitivity, with the ability to detect a single cell amount of gDNA.

References

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