Application Note

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Quality Control for Agilent SureSelect QXT WGS Library Preparation

Author

Eva Graf

Agilent Technologies, Inc.
Waldbronn, Germany

Abstract

This application note follows the Agilent SureSelect QXT WGS library preparation protocol, and demonstrates the performance of the Agilent 4200 TapeStation system for sample quality control (QC) in the workflow. The Genomic DNA ScreenTape assay is a reliable tool for serial quantitation of genomic DNA starting material, and provides integrity information. The Agilent High Sensitivity D5000 ScreenTape assay enables sizing and quantitation of the amplified indexed libraries before sequencing. The data also demonstrate that the results obtained with these DNA ScreenTape assays are comparable to information collected from the Agilent 2100 Bioanalyzer system and the Qubit Fluorometer.

Introduction

The Agilent SureSelect QXT Whole Genome library preparation kit generates libraries for Illumina paired-end multiplexed sequencing. SureSelect QXT library preparation is sensitive to input variations in genomic DNA (gDNA). The protocol recommends quantitation with two serial fluorometric assays using the Qubit instrument. This performance was compared to the same samples run on the Genomic DNA ScreenTape assay with the Agilent 4200 TapeStation system. Deviations in gDNA input amount result in suboptimal fragmentation, leading to overrepresentation of large or small DNA fragments in the amplified library. This effect was illustrated by the generation of additional libraries with a wide range of gDNA starting material, from low to high abundance.

The amplified libraries were analyzed using the Agilent High Sensitivity D5000 ScreenTape assay with the 4200 TapeStation system, and compared to the results of the High Sensitivity DNA Assay with the Agilent 2100 Bioanalyzer system that is recommended in the SureSelect QXT protocol.

The analysis of the amplified libraries revealed that the High Sensitivity D5000 ScreenTape assay matches the sizing and molarity results obtained from the 2100 Bioanalyzer system

Materials and Methods

4200 TapeStation system (G2991AA), 2100 Bioanalyzer system (G2939AA), SureCycler 8800 Thermal Cycler (G8800A), SureSelect QXT library prep kit for WGS (G9682A), High Sensitivity D5000 ScreenTape (5067-5592) with reagents (5067-5593), Genomic DNA ScreenTape (5067-5365) with reagents (5067-5366), High Sensitivity DNA Kit (5067-4626), and OneSeq Reference DNA Male (5190-8848) were obtained from Agilent Technologies. NanoDrop 1000, Qubit 3.0 Fluorometer (Q33216), and Qubit dsDNA BR Assay Kit (Q32850) were obtained from Thermo Fisher Scientific. Quantity determination of DNA libraries was measured using the region functionality. Unless stated, the manufacturer's protocols and guidelines were followed.

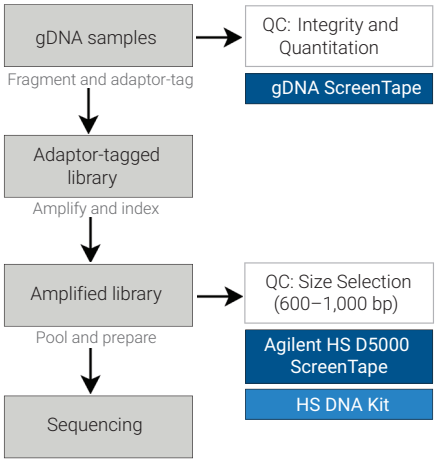

Figure 1. Workflow for Agilent SureSelect QXT WGS library preparation with quality control steps.

Results and Discussion

Figure 1 illustrates the SureSelect QXT protocol for the generation of whole-genome sequencing (WGS) libraries using gDNA as the starting material. In brief, the samples undergo fragmentation and adaptor-tagging in a single enzymatic step, followed by PCR amplification, during which the adaptor-ligated fragments are amplified and indexed. The amplified adaptor-ligated libraries are then cleaned using magnetic beads, and analyzed for proper sizing, quantitation, and purity evaluation (absence of adaptor-dimer products) before sequencing.

Quantity and integrity of gDNA

The SureSelect QXT WGS protocol requires high-quality DNA samples for optimal performance and precise quantitation of the gDNA starting material. Serial quantitation was carried out using the Qubit instrument with the dsDNA BR Assay according to the protocol. The same samples were run on the Genomic DNA ScreenTape assay with the 4200 TapeStation system and the NanoDrop with six replicates on each instrument. Figure 2 presents data from the 4200 TapeStation system, Qubit, and NanoDrop, showing the applicability of the Genomic DNA ScreenTape assay for quantitating gDNA starting material. The measurement of gDNA with UV spectroscopy tends to overestimate the quantity due to other buffer components that may absorb in the UV spectrum[4].

In addition, the Genomic DNA ScreenTape assay provides objective assessment of sample integrity within the same QC step. Sample integrity is determined automatically by the DNA integrity number (DIN) calculation provided by the TapeStation Analysis software (Figure 3).

In contrast to the other systems, the Genomic DNA ScreenTape assay with the 4200 TapeStation system offers both quality and quantity assessment in a single step from only 1 µL of sample.

Sizing of Agilent SureSelect QXT whole genome libraries

In addition to the initial QC of gDNA starting material, the SureSelect QXT protocol recommends quality control of the amplified library to ensure representation of the whole fragment size range prior to sequencing. An average fragment size significantly less than 600 bp may indicate too little gDNA in the fragmentation reaction, and may be associated with increased duplicates in the sequencing data. Conversely, libraries with an unusually large average fragment size (exceeding 1,000 bp) may indicate too much gDNA in the fragmentation reaction, and may require higher DNA concentrations for optimal cluster density in the sequencing reaction.

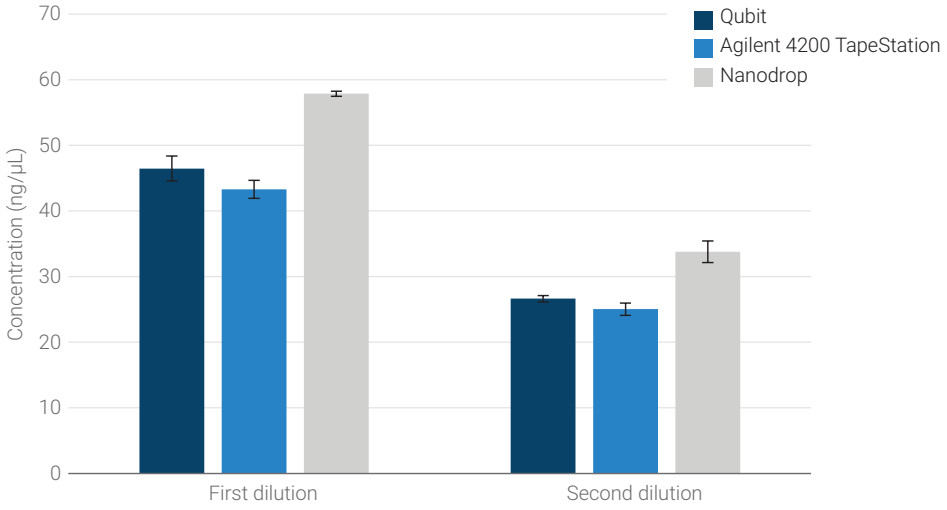

Figure 2. Quantitation data from an Agilent Genomic DNA ScreenTape assay compared to Qubit and NanoDrop.

To demonstrate this effect, libraries from 20, 50, and 80 ng input amounts were prepared with a standard library generation represented by the 50 ng gDNA sample. Figure 4 presents the performance of the 4200 TapeStation system in sizing assessed by region analysis of the libraries in triplicate from each of the above input amounts in comparison to the 2100 Bioanalyzer system. The data in the figure exhibit good correlation in sizing of the libraries by the two systems. The electropherogram overlays of the variable amounts of input gDNA show the differential effects on the smear profile of the library fragment size distribution (Figure 5) with the 2100 Bioanalyzer (A) and the 4200 TapeStation (B) systems. An optimal gDNA input amount of 50 ng displays a distinctive library curve with an

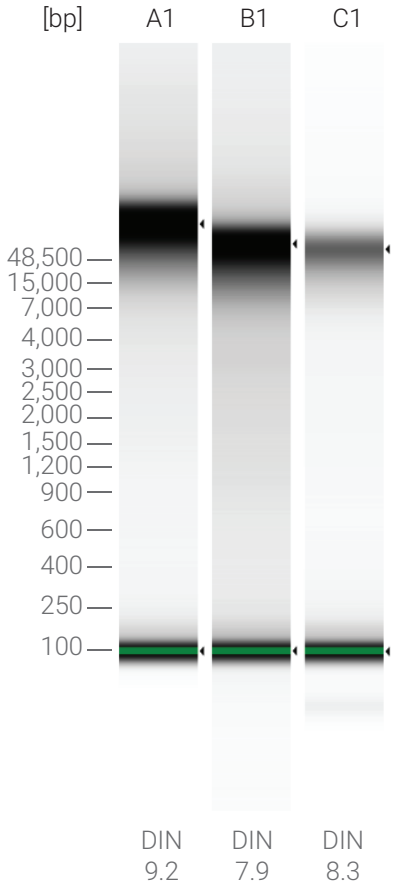

Figure 3. Gel image of genomic DNA input samples analyzed with the Agilent Genomic DNA ScreenTape assay. The gel image shows that the samples are intact, which is represented by the DNA integrity number (DIN) below the gel lines.

average size between 600 and 1,000 bp. Libraries generated from minimal gDNA input typically lead to a shift to smaller size ranges. Conversely, excess input of gDNA leads to overrepresentation of large DNA fragments, resulting in a pattern shifted to higher size ranges. Moreover, all electropherograms exhibit absence of adaptor-dimer products, indicating high library purity.

Quantitation of SureSelect QXT whole genome libraries

For multiplex sequencing, the SureSelect QXT whole genome libraries are combined such that each index-tagged sample is present in equimolar amounts in the pool. The optimal seeding concentration for libraries depends on the sequencing platform. It may also need to be optimized based on the DNA fragment size range for the library and the desired output and data quality. The 4200 TapeStation and 2100 Bioanalyzer systems provide molarity quantitation data along with sizing information in the region table (Figure 6).

For each library generated by various gDNA input amounts, the molarity was plotted in a graph comparing both systems (Figure 7). The data summarized in Table 1 demonstrate that sizing and quantitation of amplified libraries with the High Sensitivity D5000 ScreenTape assay match the results of the High Sensitivity DNA assay of the 2100 Bioanalyzer system that is recommended by the SureSelect QXT WGS protocol.

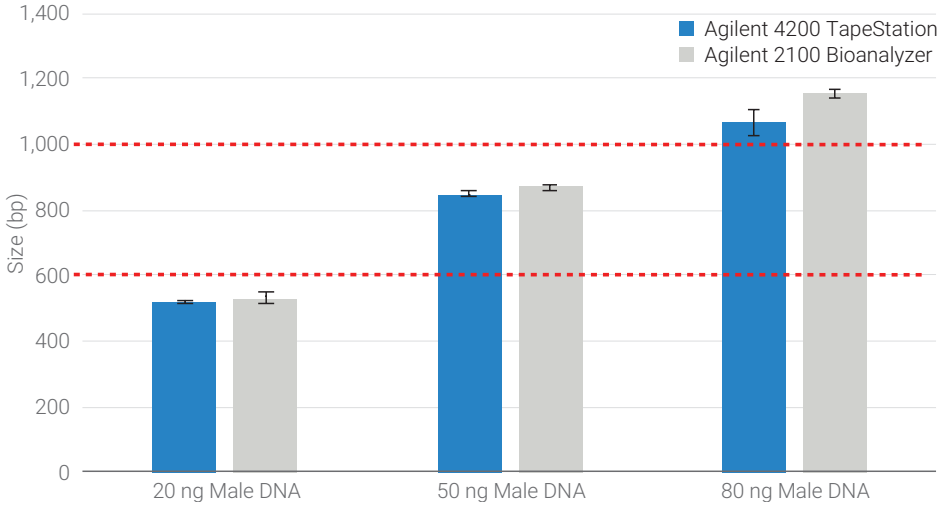

Figure 4. Sizing comparison between the Agilent 4200 TapeStation and Agilent 2100 Bioanalyzer systems. The average sizing was compared for libraries with 20, 50 (recommended), and 80 ng gDNA input amounts. Dashed lines show the borders of acceptable average size ranges for amplified libraries (600 bp and 1,000 bp).

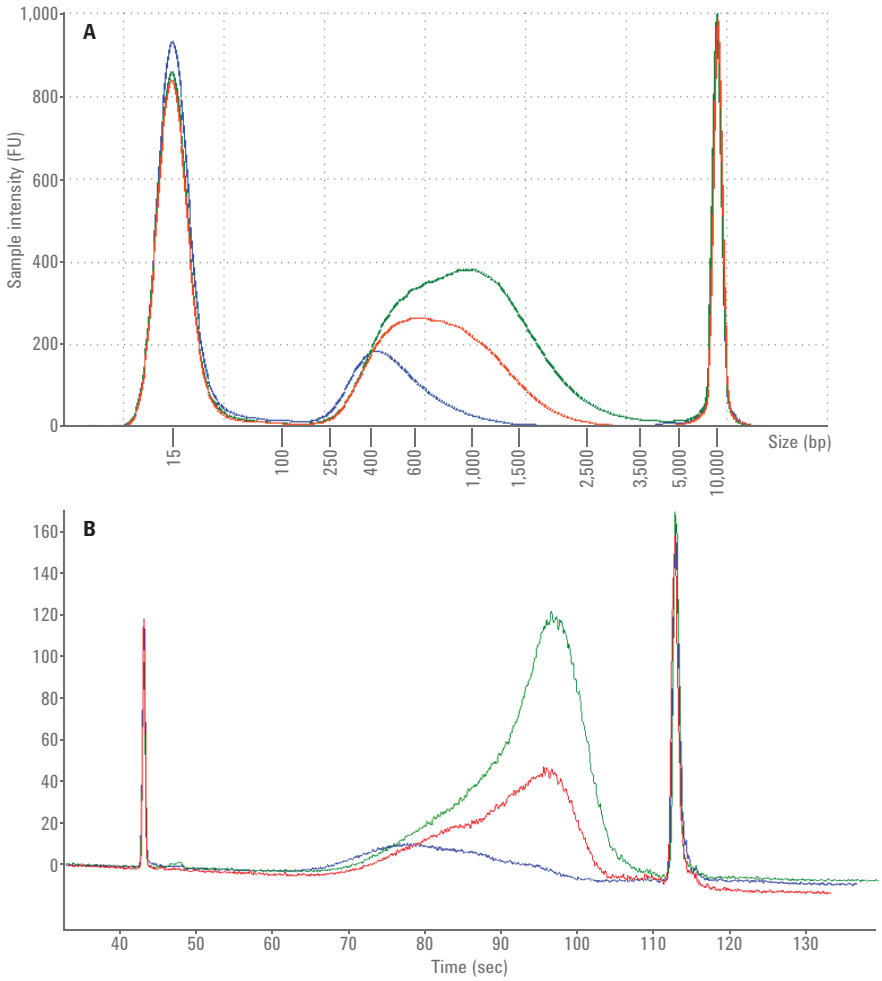

Figure 5. Electropherogram overlays from the Agilent 4200 TapeStation (A) and Agilent 2100 Bioanalyzer systems (B) of amplified libraries with 20 (blue), 50 (recommended, red), and 80 ng (green) gDNA input amounts.

Conclusion

This application note demonstrates that the Agilent 4200 TapeStation is a reliable system for sample analysis during Agilent SureSelect QXT WGS library preparation. High-quality DNA samples and reproducible quantitation of the gDNA starting material are indispensable for successful SureSelect QXT library preparation. The Agilent Genomic DNA ScreenTape assay ensures precise quantitation of genomic DNA starting material, and provides assessment of sample integrity within the same QC step. The Agilent High Sensitivity D5000 ScreenTape assay is an ideal tool for sizing and quantitation of SureSelect QXT WGS amplified libraries. In addition to its equivalent performance to the Agilent 2100 Bioanalyzer system, which is recommended in the SureSelect QXT protocol, the 4200 TapeStation system offers a highly flexible sample throughput and ease of use.

A

Region Table | Sample Table

From [bp]	To [bp]	Average Size [bp]	Conc. [pg/µl]	Region Molarity [pmol/l]
250	2500	855	667	1510

B

	From [bp]	To [bp]	Average Size [bp]	Conc. [pg/µl]	Molarity [pmol/l]
1	250	2,700	856	631.72	1,514.6

Results | Peak Table | Region Table | Legend

Figure 6. Screenshots of region tables presenting average size, concentration, and molarity of amplified libraries. A) Agilent TapeStation Analysis software in region view. B) Agilent 2100 Expert software with smear analysis activated.

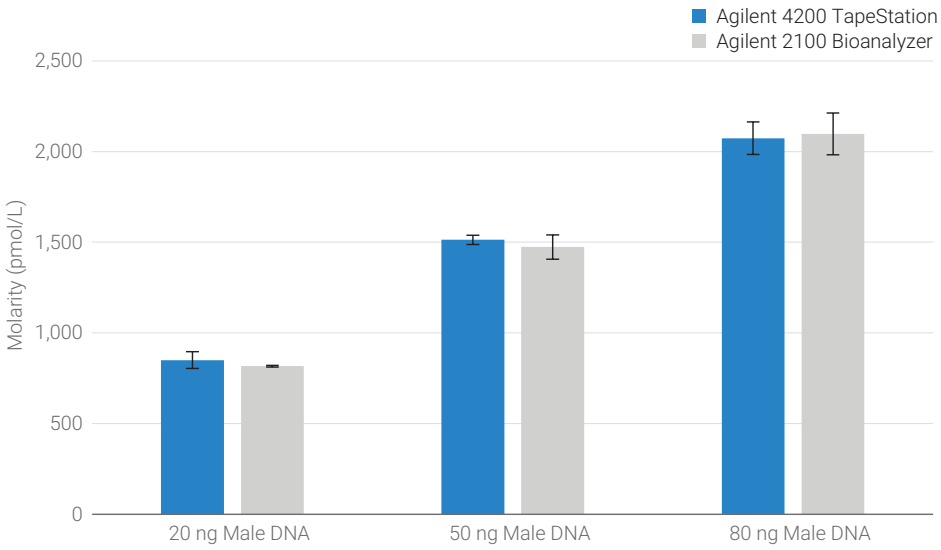

Figure 7. Quantitation of amplified libraries compared between the Agilent 4200 TapeStation and Agilent 2100 Bioanalyzer systems.

Table 1. Sizing and quantitation results of amplified libraries analyzed with the Agilent 4200 TapeStation and Agilent 2100 Bioanalyzer systems.

		Average size (bp)		Region molarity (pmol/L)	
Starting material		Agilent 4200 TapeStation System	Agilent 2100 Bioanalyzer System	Agilent 4200 TapeStation System	Agilent 2100 Bioanalyzer System
20 ng	mean	519	533	850	817
	% CV	1.2	2.8	5.4	0.6
50 ng	mean	849	868	1513	1473
	% CV	0.6	1.4	1.7	4.5
80 ng	mean	1065	1157	2073	2097
	% CV	3.8	1.1	4.3	5.5

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