

Improving sample quality for target enrichment and next-gen sequencing with the Agilent High Sensitivity DNA Kit and the Agilent SureSelect Target Enrichment Platform

Application Note

Genomics

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Abstract

Next-generation sequencing (NGS) has revolutionized the genetic landscape. It is a lengthy, labor-intensive process that yields results never before achieved. As a result, it is imperative that the quality of the DNA sample be evaluated from the start, as most NGS sample preparation protocols require PCR amplification to generate DNA libraries prior to sequencing. The likelihood of artifact generation could contribute to bias, affecting the potential results. The High Sensitivity DNA Kit used with the Agilent 2100 Bioanalyzer has been optimized with improved levels of detection. The improved sensitivity allows the numbers of library PCR cycles to be reduced, removing amplification bias and significantly improving the quality of NGS data with increased accuracy.

This Application Note describes how the Agilent 2100 Bioanalyzer High Sensitivity DNA Kit can be used to provide quantitative and qualitative information about the DNA samples used in the Agilent SureSelect Target Enrichment System.



Agilent Technologies

Introduction

The Agilent 2100 Bioanalyzer, an automated on-chip electrophoresis system, has already proven to be a valuable tool for automated sizing and quantification of various double-stranded DNA sample types relevant for the next-generation sequencing (NGS) sample preparation workflow.¹

The Agilent 2100 Bioanalyzer with the DNA 1000 kit is recommended by NGS platform providers for measuring DNA sample quality prior to sequencing runs. These quality checks reduce time and resources wasted by low-quality samples. Recently, a High Sensitivity DNA kit was developed which offers improved sensitivity for checking the size and quantity of precious low concentrated DNA starting material or DNA libraries down to a concentration of 100 pg/ μ L.

Next-generation sequencing technology has brought high throughput to genome sequencing, but the new processes lack the ability to target specific areas of a genome. The SureSelect Target Enrichment System, enables genomic areas of interest to be sequenced exclusively. This creates process efficiencies that reduce costs and allow more samples to be analyzed per study.² The Agilent High Sensitivity DNA Kit and the Agilent 2100 Bioanalyzer can be used for quality control at several steps during the SureSelect Target Enrichment workflow. During the sample preparation, the Agilent 2100 Bioanalyzer is used for quality control and sizing selection

of the sheared genomic DNA, and to assess the quality and size distribution of the PCR amplified sequencing library DNA. After post-hybridization amplification, the Agilent 2100 Bioanalyzer can be used to determine the quality and the concentration of the PCR-amplified capture DNA before sequencing.

This Application Note describes how the High Sensitivity DNA kit and the Agilent 2100 Bioanalyzer can be used before sequencing to reduce the number of required PCR cycles. This reduces amplification bias, thus improving the quality of DNA libraries created during the SureSelect Target Enrichment workflow.

Experimental

DNA library preparation

The DNA library was prepared for Illumina's Genome Analyzer II sequencers according to manufacturer's instructions.

SureSelect Target Enrichment

The SureSelect Target Enrichment for the Illumina single-end sequencing platform, consisting of three main steps; sample preparation, hybridization and post hybridization amplification, was carried out as described in the manual.³ 16 DNA samples obtained after the post-hybridization amplification with different numbers of PCR cycles (4-18) were used for DNA analysis with the Agilent 2100 Bioanalyzer.

High Sensitivity DNA analysis with the Agilent 2100 Bioanalyzer

The on-chip DNA electrophoresis was performed on the Agilent 2100 Bioanalyzer in combination with the Agilent High Sensitivity DNA kit, according to the High Sensitivity DNA kit guide.⁴ A dedicated High Sensitivity DNA assay is available with the Agilent 2100 Expert software (revision B.02.07 or higher). An integration region from 100 to 2000 bp was used for all samples for smear quantification.

DNA quantification

In addition to the fluorescence-based DNA quantification on the Agilent 2100 Bioanalyzer, the Qubit fluorometer and the Qubit Quant-iT dsDNA BR Assay kit were used for DNA quantification according to the manufacturer's instructions.

Results and discussion

This Application Note describes how the Agilent High Sensitivity DNA kit and the Agilent 2100 Bioanalyzer can be used to further improve the quality of DNA sequencing libraries enriched by the SureSelect kit. For this purpose, 16 amplified and purified DNA samples from the post-hybridization PCR amplification step were analyzed with the High Sensitivity DNA kit and the 2100 Bioanalyzer prior to sequencing on the Illumina platform.

Figure 1 shows electropherograms of typical PCR amplified DNA libraries. The electropherograms show a typical smear from 150 to 350 nucleotides. The primers/primer-dimers migrated very close to the lower marker, but did not affect the analysis. The excellent sensitivity of the High Sensitivity DNA kit allowed the amplified DNA to be detected and reliably quantified, even after only four PCR cycles (figure 2). As expected, DNA concentration increased with the number of PCR cycles. Above 14 PCR cycles, the increase in DNA concentration was no longer linear and becomes saturated (figure 1B). When using 10 or more cycles, the DNA concentration was outside the quantitative range of the High Sensitivity assay. These samples were diluted with TE buffer in the indicated dilution ratios (figure 1B) prior to the analysis on the Agilent 2100 Bioanalyzer.

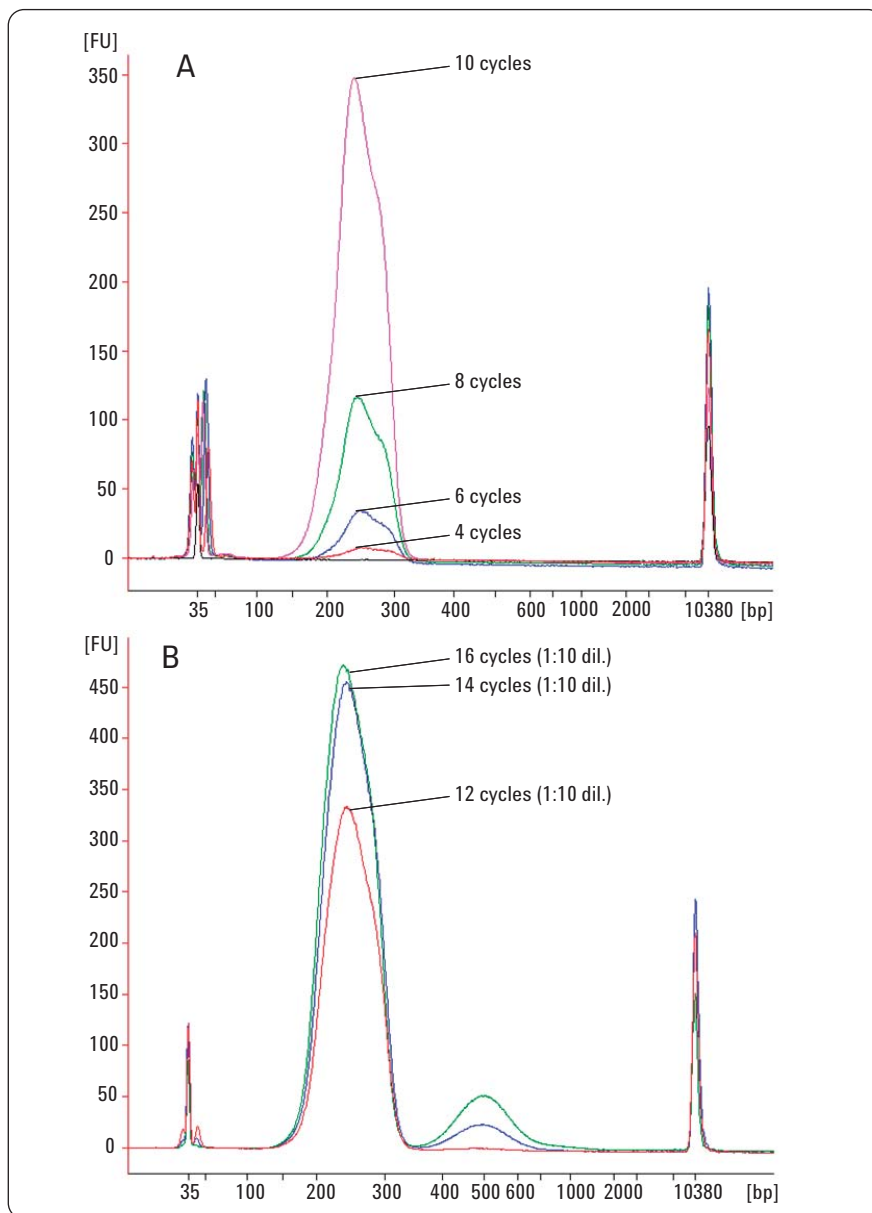


Figure 1
PCR-amplified DNA library derived from the SureSelect Target Enrichment workflow, analyzed with the High Sensitivity DNA kit.
(A) Overlay of DNA electropherograms obtained after 4 to 10 PCR cycles as well as TE buffer blank (black). The number of PCR cycles is indicated in the electropherogram overlay.
(B) Overlay of DNA electropherograms obtained after 12 to 18 PCR cycles. The number of PCR cycles and the dilution ratios are indicated in the electropherogram overlay.

The key observation clearly shown in figure 1B, is that the quality of the PCR product depended on the number of PCR cycles performed. After 14 PCR cycles, an additional DNA smear at approximately 500 bp was detected in the electropherogram. This PCR artifact could potentially affect the efficiency of an NGS experiment. When running amplifications with PCR cycles below 14 this PCR artifact was not observed. DNA library analysis with the High Sensitivity DNA kit allows the number of required PCR cycles to be reduced. This results in fewer amplification-related artifacts, significantly improving the DNA sample quality for downstream sequencing. It should be expected that the improved DNA sample quality due to the reduced number of PCR cycles will positively affect the outcome of the downstream sequencing by reducing allelic bias, single-stranded DNA generation, and duplicate sequences.⁵

The DNA concentrations determined with the High Sensitivity DNA kit were also compared with the DNA concentrations measured with a fluorometer (table 1). The DNA samples obtained after 4 to 10 PCR cycles were measured directly without dilution; all other samples were diluted as indicated in figure 1. The selected assay on the fluorometer permitted DNA quantification only after 10 or more PCR cycles. The High Sensitivity DNA kit provided a reproducible DNA quantification after only four PCR cycles. For added confidence in our results, each DNA sample was measured four times on two different High Sensitivity DNA chips with the Agilent 2100 Bioanalyzer.

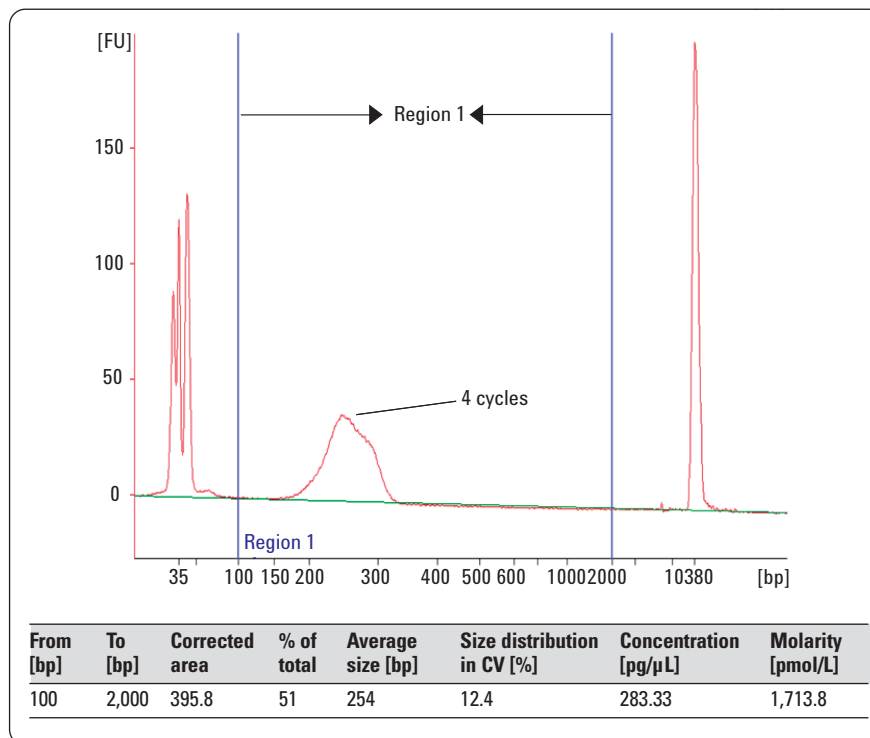


Figure 2
Electropherogram obtained after 4 PCR cycles. The integration region from 100 to 2000 bp was used for smear quantification.

PCR cycles	Fluorometer DNA conc. [ng/μL]	2100 Bioanalyzer DNA conc. [ng/μL]	Deviation from fluorometer [%]
4	-	0.090 ± 0.015	-
	-	0.083 ± 0.007	-
6	-	0.304 ± 0.017	-
	-	0.301 ± 0.010	-
8	-	1.13 ± 0.07	-
	-	1.34 ± 0.07	-
10	3.4	4.49 ± 0.16	32.1
	3.4	4.54 ± 0.17	33.8
	3.3	3.68 ± 0.22	10.3
	4.1	4.03 ± 0.23	-1.0
12	13.4	14.5 ± 0.5	8.0
	12.5	13.9 ± 0.6	11.8
14	36.4	39.8 ± 3.5	9.4
	43.6	51.6 ± 3.3	18.3
18	51.0	63.7 ± 3.4	24.9
	61.4	70.0 ± 5.4	14.0

Table 1
Comparison of DNA quantification with a fluorometer and the Agilent 2100 Bioanalyzer. 16 different DNA samples obtained from the post-hybridization amplification step of the SureSelect Target Enrichment workflow were quantified with the fluorometer and the Agilent 2100 Bioanalyzer. The DNA samples were measured directly (4 to 10 PCR cycles) or after dilution (12 to 18 cycles) as indicated in figure 1. The standard deviation for the total DNA concentration measured with the Agilent 2100 Bioanalyzer was calculated from four data points measured on two different DNA chips.

Above 10 PCR cycles, overall DNA concentrations are generally similar for both methods. Variances in the DNA concentration determined with the fluorometer and the on-chip electrophoresis could be due to the differences in technologies. Different fluorescent dyes are used, and the quantification by the Agilent 2100 Bioanalyzer is preceded by an electrophoretic separation of the sample. Additional variances were introduced by diluting the samples.

Figure 3 graphically summarizes the data from table 1. The DNA concentrations obtained through both DNA quantification methods were plotted against the number of PCR cycles. Both methods, the fluorometer and the on-chip electrophoresis, clearly show the expected sigmoid amplification rate. The results obtained with both methods are in good agreement with each other. The Agilent 2100 Bioanalyzer provides DNA quantification as well as additional valuable information on the quality of the enriched DNA library.

The insert in figure 3 shows the same data in double logarithmic scale to demonstrate the linearity of both methods. The linear dynamic range for the SureSelect DNA samples analyzed with the High Sensitivity DNA assay and the Agilent 2100 Bioanalyzer was determined to be 80 to 5000 pg/ μ L with $r^2= 0.9996$. The last data point, after 18 PCR cycles, was not taken into account for this linearity analysis, as the PCR begins to saturate after 14 cycles.

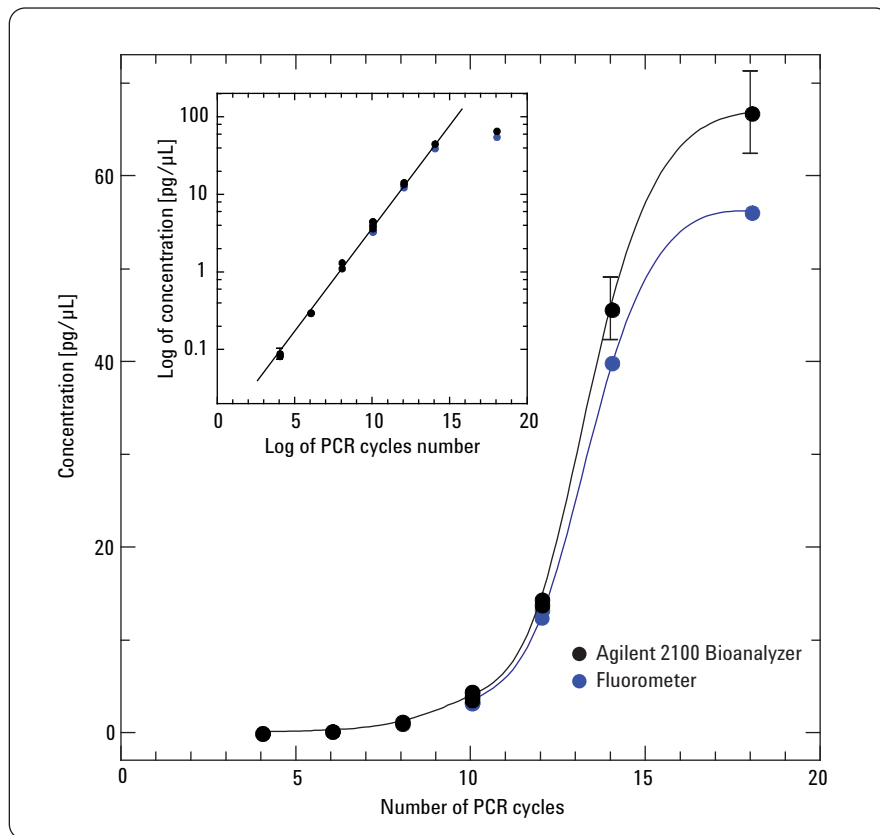


Figure 3 Comparison of DNA quantification with a fluorometer and the Agilent 2100 Bioanalyzer. The obtained DNA concentrations (table 1) were plotted against the number of PCR cycles, Agilent 2100 Bioanalyzer (● black), fluorometer (● blue). The insert shows the same data in double logarithmic scale to demonstrate the linearity of both methods.

Conclusion

Quality control of DNA samples after library generation derived from the SureSelect Target Enrichment workflow can easily be performed with the High Sensitivity DNA kit and the Agilent 2100 Bioanalyzer.

The High Sensitivity DNA kit offers increased sensitivity for DNA analysis down to pg/ μ L concentrations across a broad linear dynamic range. This enhanced performance allows the number of required PCR cycles to be significantly decreased, eliminating PCR artifacts, while still reliably quantifying the sample. The improved DNA quality will improve the outcome of downstream sequencing analysis, maximizing throughput efficiency while minimizing cost per sample

Therefore, the number of required PCR cycles can be significantly decreased, eliminating PCR artifacts, while still reliably quantifying the sample.

References

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