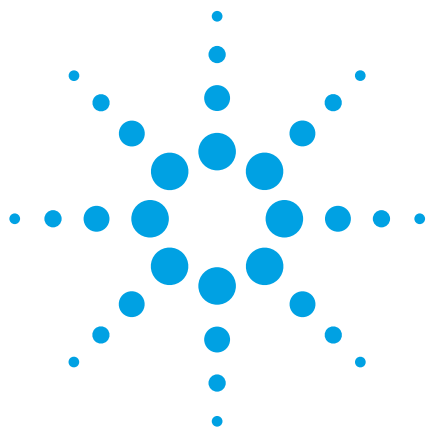
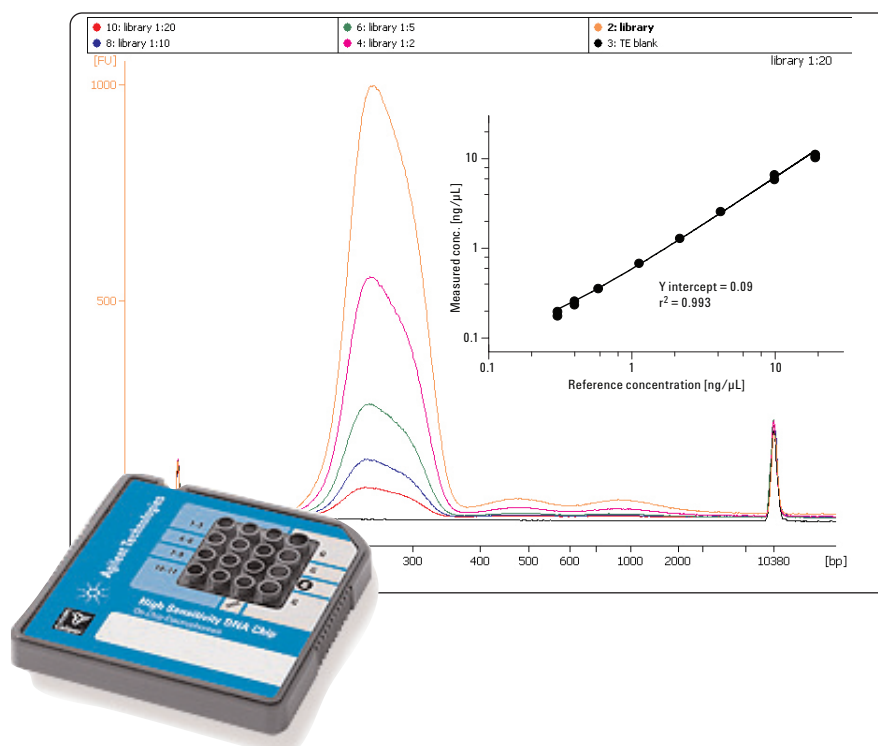




Performance characteristics of the High Sensitivity DNA kit for the Agilent 2100 Bioanalyzer

Technical Note



Introduction

This publication demonstrates the performance of the Agilent High Sensitivity DNA kit for sizing, quantification, and quality control of double-stranded DNA fragments and complex samples, such as DNA sequencing libraries or fragmented DNA. The kit offers:

- Increased sensitivity for DNA analysis down to pg/μL sensitivity
- Large size range from 50–7000 bp
- Broad linear dynamic range – 2 orders of magnitude
- Easily defined smear regions with detailed data analysis



Agilent Technologies

Overview

This Technical Note describes the performance of the Agilent High Sensitivity DNA kit for high sensitivity, on-chip DNA sizing and quantification. This DNA kit further extends the DNA product portfolio for sizing, quantification and quality control of DNA with the Agilent 2100 Bioanalyzer. The DNA 1000, DNA 7500 and DNA 12000 kits allow the user to automatically size and quantitate polymerase chain reaction (PCR) and reverse transcription (RT)-PCR products in single or multiplex mode, and restrict digests accurately and reproducibly. The High Sensitivity DNA kit was developed for sizing and quantification of DNA fragments and DNA smears in the 50 to 7000 bp size range down to pg/ μ L sensitivity. It is especially designed for sample quality control and the monitoring of critical steps in next generation sequencing (NGS) workflows, including DNA fragmentation, target enrichment, and DNA library amplification. Many NGS sample preparation protocols require DNA library amplification by PCR which can often introduce amplification bias. The increased detection sensitivity of the High Sensitivity DNA kit significantly lowers the number of required PCR cycles, reducing amplification artifacts and improving the quality of sequencing data. With the increased use of NGS platforms, sizing, quantification and quality assessment of fragmented DNA starting material and DNA sequencing libraries has become essential to achieving high quality sequencing data. The Agilent 2100 Bioanalyzer is recommended by NGS platform providers for the automated sizing and quantification of various sample types relevant for the NGS sample preparation workflow.

Experimental

Materials and equipment

Agilent High Sensitivity DNA kit (Agilent part number 5067-4626), DNA 1000 kit (Agilent part number 5067-1504), and DNA 7500 kit (Agilent part number 5067-1506), Agilent 2100 Bioanalyzer (Agilent Technologies Waldbronn, Germany). Typical DNA samples were kindly provided by Sanger Institute (Cambridge, UK), CRUK Cambridge Research Institute (Cambridge, UK), and GATC-Biotech (Konstanz, Germany).

High sensitivity DNA analysis with the Agilent 2100 Bioanalyzer

The chip-based separations were performed on the Agilent 2100 Bioanalyzer in combination with the Agilent High Sensitivity DNA kit in comparison to the DNA 1000 and 7500 kits. A dedicated High Sensitivity DNA assay is available within the Agilent 2100 Expert software (revision B.02.07 or higher). The on-chip electrophoresis was per-

formed according to the High Sensitivity DNA kit guide.¹ The kit includes 10 chips for a total of 110 samples, DNA ladder, marker, reagents and a Quick Start Guide.²

Results and discussion

DNA smear analysis

The Agilent 2100 Expert software (version B.02.07 or higher) offers a significantly improved smear analysis with an easy-to-use integrator (Figure 1), which is available for the High Sensitivity DNA assay and all other DNA assays. The improved smear identification provides sliders within the electropherogram that allow size adjustments of the smear region. One adjustable smear region is predefined in the Agilent 2100 Expert software, but additional regions can be added. The software automatically determines the average size (bp), size distribution in CV (%), concentration (pg/ μ L), % of total, and molarity (pmol/L) for each defined smear region. The region table displayed underneath the electropherogram summarizes the smear results of all regions per sample.

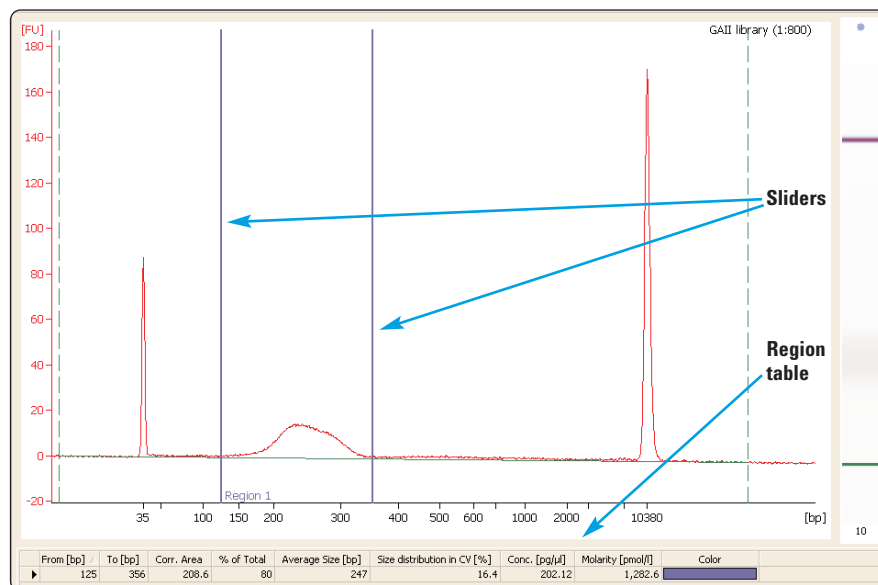


Figure 1
DNA smear analysis with the 2100 Expert software. Multiple regions can be defined within the 2100 Expert software; sliders allow adjusting the size range. The % of total, average size, concentration and molarity are automatically determined and displayed in the region table.

Sizing range

Figure 2A shows a comparison of the sizing range of the High Sensitivity DNA kit to the DNA 1000 and the DNA 7500 kits. For this comparison the DNA ladders of the three different DNA kits were analyzed using the corresponding DNA assays. The sizing range of the High Sensitivity DNA kit is enlarged compared to the DNA 1000 and 7500 kits covering size ranges of 25–1000 bp and 100–7500 bp, respectively. The High Sensitivity DNA assay allows the analysis of DNA in the size range of

50–7000 bp (Table 1), which almost completely covers the size range of the DNA 1000 and 7500 kit.

The analysis of fragmented DNA with the DNA 1000 and the High Sensitivity DNA kits (Figure 2B) clearly demonstrates the benefits of this increased size range for the targeted NGS applications. The fragmented DNA (3.1 ng/μL) is clearly separated from the upper and the lower marker with the High Sensitivity DNA kit, allowing a correct sizing and quantification analysis.

When the same sample in a higher DNA concentration is analyzed with the DNA 1000 kit, the upper marker overlaps with the sample. Therefore, a correct sizing and quantification analysis is not possible.

The special size range from 50–7000 bp allows the analysis of larger fragmented DNA smears, well defined DNA libraries, and DNA adaptors of small sizes with a single kit.

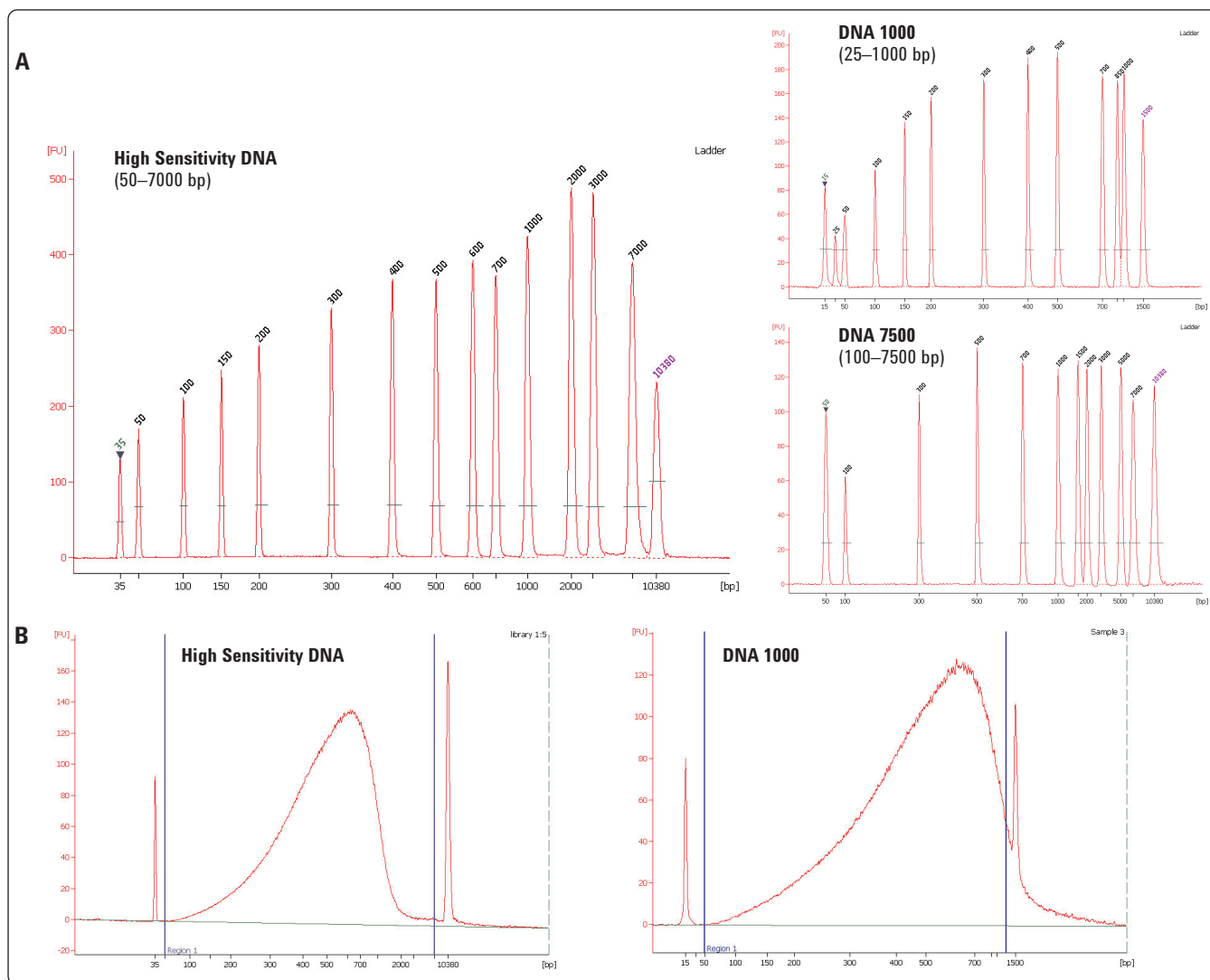


Figure 2
Size range of the High Sensitivity DNA kit. (A) Shows a size range comparison to the DNA 1000 and 7500 kit analyzing the corresponding DNA ladders. (B) Demonstrates the benefits of the extended size range when analyzing fragmented DNA. 3.1 ng/μL were analyzed with the High Sensitivity DNA kit and 230 ng/μL with the DNA 1000 kit.

Sizing accuracy and reproducibility

To determine the sizing accuracy and reproducibility of the High Sensitivity DNA kit, the ladder was run on different chips. The High Sensitivity DNA ladder contains 13 different DNA fragments from 50 to 7000 bp. For each DNA ladder fragment, n = 70 was considered to determine the sizing accuracy and reproducibility. Under these experimental conditions, the sizing offset for all 13 ladder fragments was below 5 % (see the upper panel of Figure 3). The lower panel of Figure 3 demonstrates excellent sizing reproducibility. For 12 ladder fragments, the % CV was below 2.5 and only the 3000 bp fragment had a % CV slightly above 3. A sizing accuracy of 10 % and a sizing reproducibility of 5 % or better are specified for the High Sensitivity DNA kit (Table 1).

High Sensitivity DNA kit	
Analytical specifications	
Sizing range	50–7000 bp
Sizing resolution	50–600 bp: ±10 % 600–7000 bp: ±20 %
Sizing accuracy	± 10 % CV*
Sizing reproducibility	5 % CV*
Quantification accuracy	20 % CV*
Quantification reproducibility	50-2000 bp: 15 % CV* 2000–7000 bp: 10 % CV*
Quantitative range	5-500 pg/μL
Maximum salt conc. in sample	10 mM Tris and 1 mM EDTA
Physical Specifications	
Analysis run time	45 minutes
Samples per chip	11
Sample volume	1 μL
Kit stability	4 months (see box for storage temp.)
Kit size	10 chips 11 DNA samples/chip = 110 samples/kit

Table 1
High Sensitivity DNA kit specifications. * High Sensitivity DNA ladder as sample.

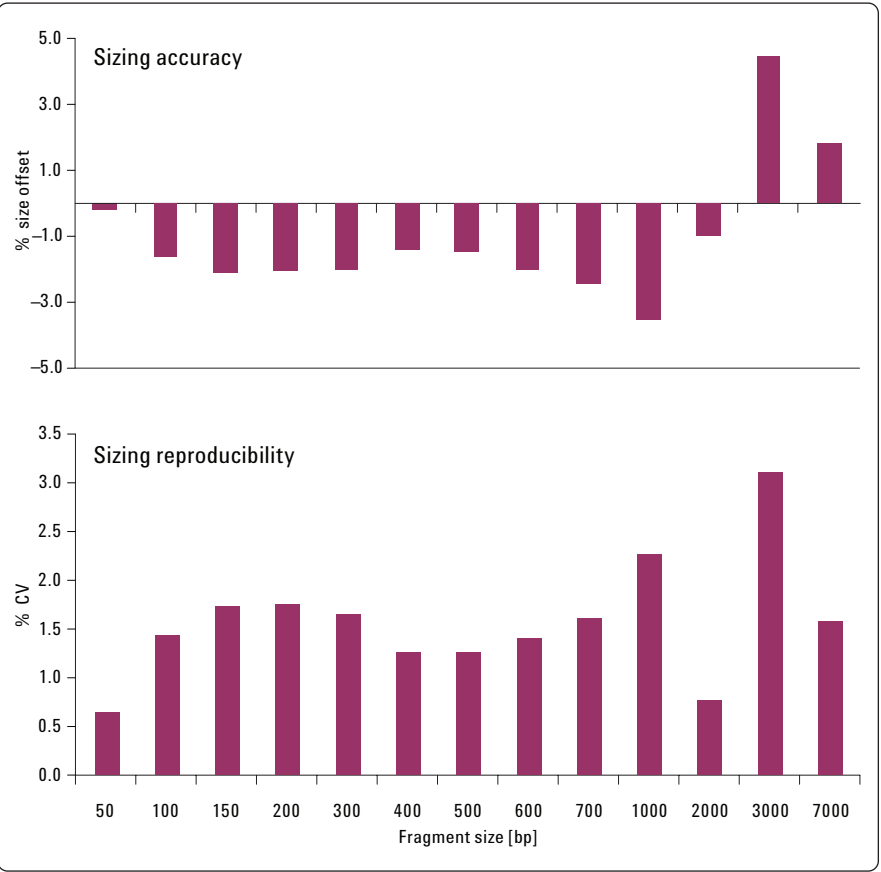


Figure 3
Sizing accuracy and reproducibility. The High Sensitivity DNA ladder was analyzed with the High Sensitivity DNA kit (n = 70 for each DNA fragment). The upper panel demonstrates the sizing accuracy (% size offset) and the lower panel the sizing reproducibility (% CV).

Sensitivity

The corresponding ladders were diluted and analyzed with the matching assays (Figure 4) to compare the sensitivity of the newly developed High Sensitivity DNA kit to the DNA 1000 kit. The High Sensitivity DNA ladder was analyzed with a DNA concentration of 5 pg/ μ L per peak and the DNA 1000 ladder was analyzed with a DNA concentration of 150 pg/ μ L per peak. When evaluating both electropherograms, a higher signal intensity of the High Sensitivity DNA kit is observed in comparison to the DNA 1000 kit. This was true even though 30 times lower DNA concentrations were analyzed.

In further experiments, the quantitative range of the High Sensitivity DNA kit was determined to be 5-500 pg/ μ L per ladder peak (Table 1). For comparison, the quantitative range of the DNA 1000 kit is 0.1-50 ng/ μ L as described previously.³ In the experiment described here, it was determined that the High Sensitivity DNA kit achieved 40 times greater sensitivity compared to the DNA 1000 kit with respect to peak area and signal-to-noise ratio. These results could be as high as 80 times greater for DNA libraries. Therefore, under certain conditions, a sensitivity higher than the specified 5 pg/ μ L can be achieved. When analyzing DNA smears, which are less clearly defined DNA fragments, detection down to 100 pg/ μ L is possible.

Linear dynamic range

Two typical samples from NGS workflows were analyzed to determine the linear dynamic range of the High Sensitivity DNA kit. Figure 5A shows the analysis of a dilution series of a DNA library obtained with the Illumina/Solexa workflow and a blank sample (10 mM Tris and 1 mM EDTA). The concentration of the initial DNA sample was determined with NanoDrop

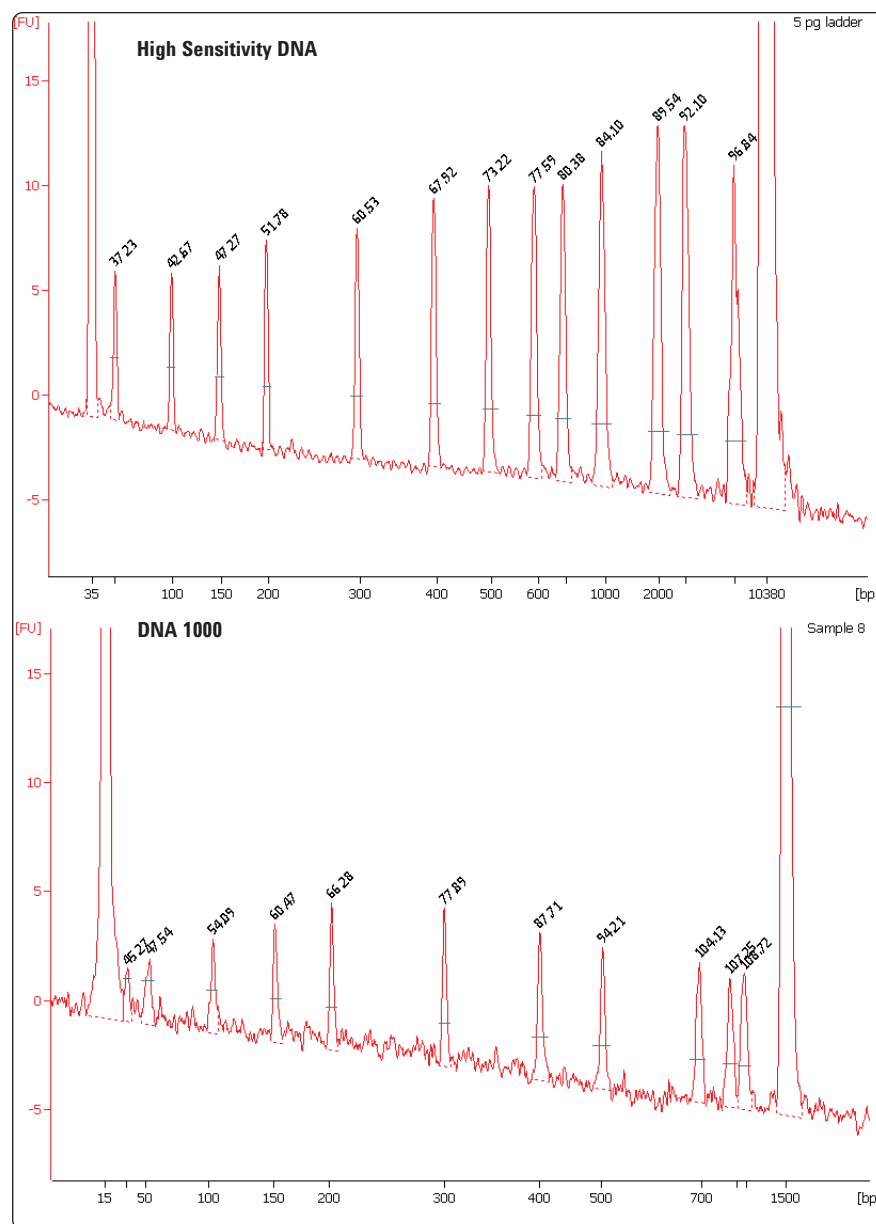


Figure 4

High Sensitivity DNA and DNA 1000 ladders were analyzed with the High Sensitivity DNA or DNA 1000 kits respectively. The zoomed electropherograms show the analysis of a DNA concentration of 5 pg/ μ L per peak with the High Sensitivity DNA and 150 pg/ μ L per peak with the DNA 1000 kit.

and slab gel electrophoresis. The DNA sample was then diluted with 10 mM Tris and 1 mM EDTA to eight reference DNA concentrations ranging from 0.23 up to 18.5 ng/ μ L. Figure 5B summarizes the results of a comparable experiment,

analyzing a dilution series of fragmented DNA. The five reference DNA concentrations ranged from 0.77 up to 15.3 ng/ μ L.

For both DNA sample types, the double logarithmic plot demonstrates an excellent linearity with $r^2 = 0.993$. In both cases the concentration of the sample with the highest DNA reference concentration, 18.5 ng/ μ L for the DNA library and 15.3 ng/ μ L for the fragmented DNA, is slightly underestimated (Figure 5). This indicates that the upper end of the linear dynamic range is prob-

ably reached and the signal starts leveling off.

In several additional experiments with a wide range of samples types, the linear dynamic range for smear samples of the High Sensitivity DNA kit was found to be between 50–100 pg/ μ L and 5000–10000 pg/ μ L. The linear dynamic range depends on the library type and

fragment distribution. DNA samples with a broad fragment distribution are at the upper end of the linear dynamic range and DNA libraries with a narrow fragment distribution are at the lower end of the linear dynamic range. The broad linear dynamic range of the High Sensitivity DNA kit enables the detection of less abundant products, such as PCR artifacts and impurities.

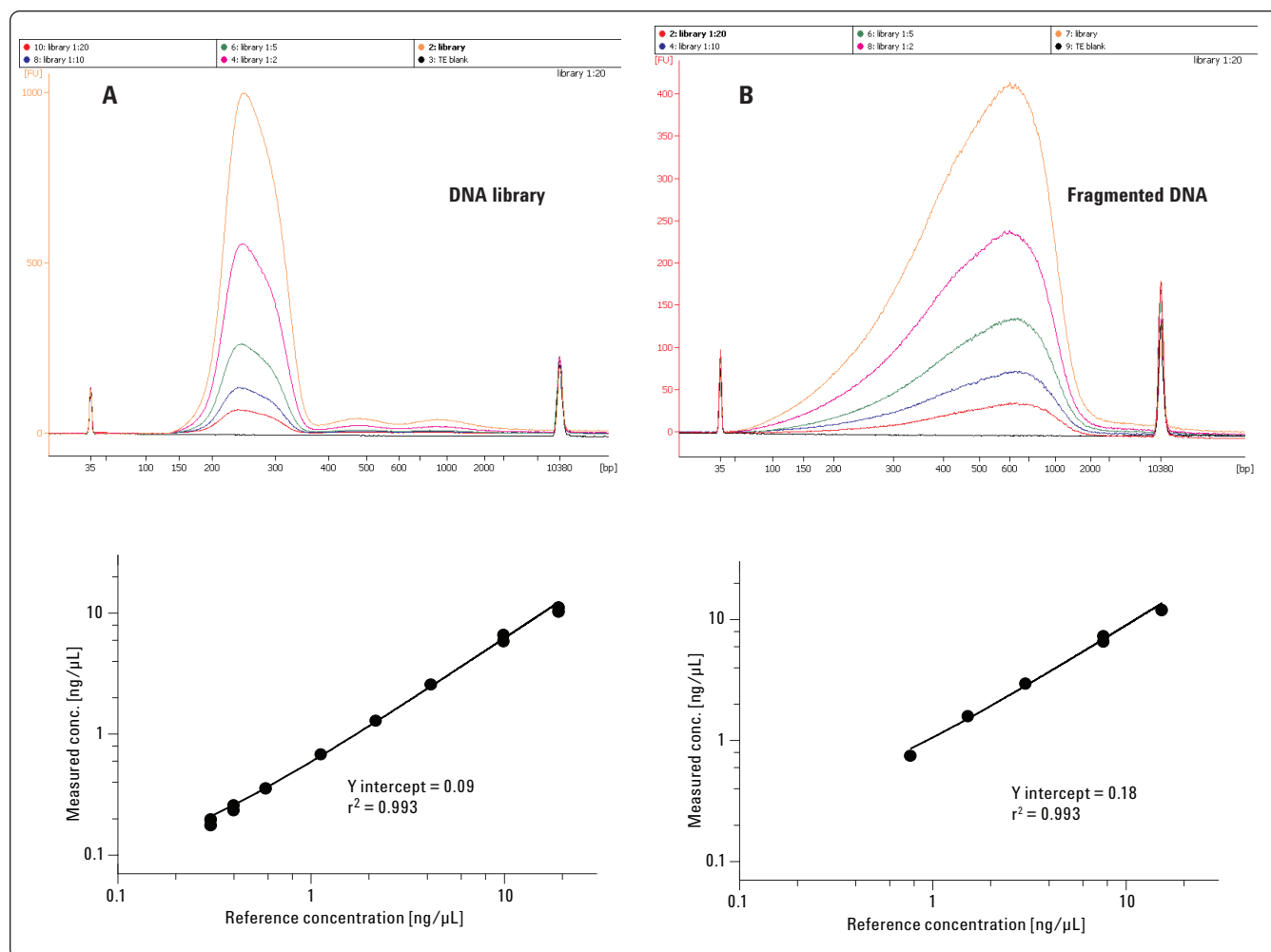


Figure 5

Linearity of the High Sensitivity DNA kit. Analysis of dilution series from two typical NGS workflows, (A) Illumina DNA library, reference DNA concentrations in ng/ μ L: 18.5 (orange), 9.25 (magenta), 3.70 (green), 1.85 (blue) and 0.93 (red), and blank (black). For easier comparison, the three lowest concentration (0.46, 0.31, and 0.23 ng/ μ L) are only included in the graph not in the electropherogram overlay. (B) Fragmented DNA, reference DNA concentration in ng/ μ L: 15.3 (orange), 7.7 (magenta), 3.0 (green), 1.53 (blue) and 0.77 (red) and blank (black).

Quantification accuracy and reproducibility

The data to determine the quantification accuracy and reproducibility of the High Sensitivity DNA kit was derived from the same chip runs used to determine sizing accuracy and reproducibility. For each of the 13 DNA ladder fragments, $n=70$ was considered.

Figure 6 shows that the obtained quantification reproducibility is below 9 % CV for all 13 ladder peaks. The High Sensitivity DNA kit is specified to deliver a minimum quantification reproducibility of 15 % CV in the 50-2000 bp size range and 10 % CV in the 2000-7000 bp size range (see Table 1).

In addition, for all 13 DNA ladder peaks, good quantification accuracy was observed with a maximum offset of below 13 % from the target concentration for the smallest ladder peak (Figure 6). Each ladder peak had a target DNA concentration of 150 pg/ μ L.

When analyzing actual samples, the quantification accuracy can be affected by differences in the sample such as the salt content. To demonstrate this effect, the High Sensitivity DNA ladder

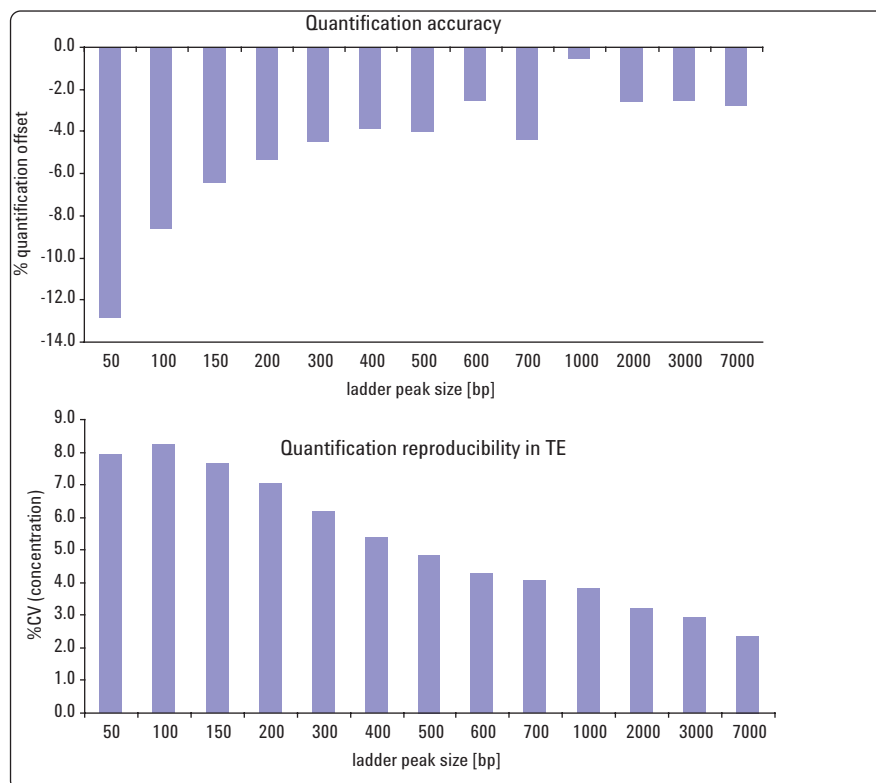


Figure 6
Quantification accuracy and reproducibility. The High Sensitivity DNA ladder was analyzed with the High Sensitivity DNA kit ($n = 70$ for each DNA fragment). The upper panel demonstrates the quantification accuracy (% quantification offset) and the lower panel the quantification reproducibility (% CV).

was measured in 10 mM Tris, 1 mM EDTA (1x TE) in the presence of different concentrations of $MgCl_2$ or KCl

(Figure 7). The increasing salt content decreases the signal intensity significantly and therefore affects quantification.

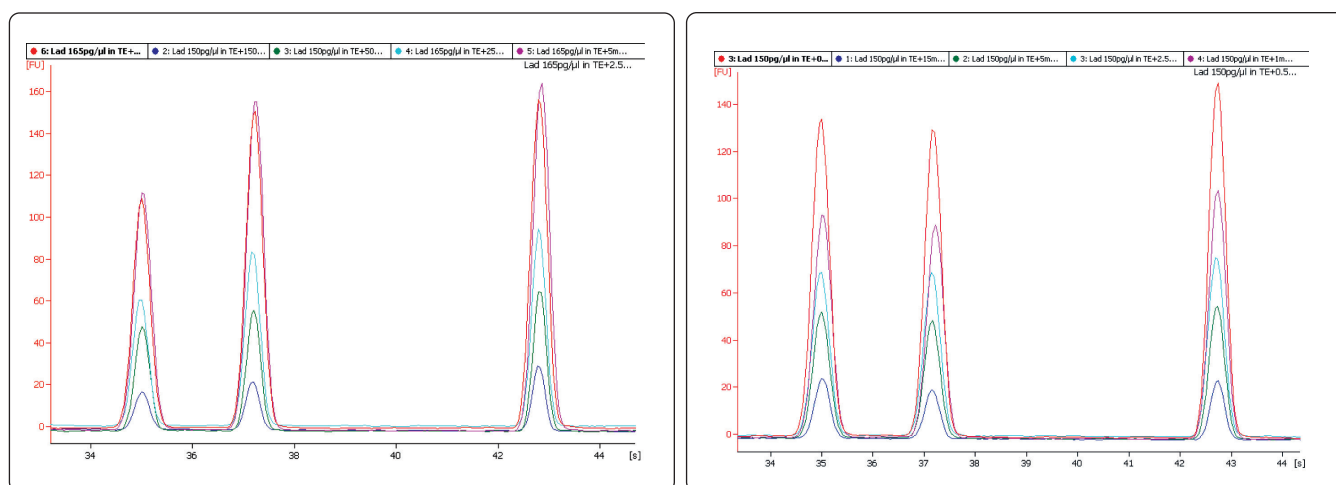


Figure 7
Salt affects DNA analysis. The High Sensitivity DNA ladder was measured in 10 mM Tris and 1 mM EDTA in the presence of different concentrations of $MgCl_2$ (right) or KCl (left). The electropherograms show lower marker (35 bp) and the first two ladder peaks at 50, and 100 bp. KCl concentrations in mM: 2.5 (red), 5 (magenta), 25 (light blue), 50 (green), 150 (blue). $MgCl_2$ concentrations in mM: 0.5 (red), 1 (magenta), 2.5 (light blue), 5 (green), 15 (blue).

Although the High Sensitivity DNA kit is salt-sensitive, and tolerates less salt than the DNA 1000 or 7500 kits, quantification accuracy is still within the 20 % CV specification if the salt content is below 5 mM KCl or 0.5 mM MgCl₂. Sizing as well as quantification reproducibility is not affected by salt in the sample buffer. However, to avoid quantification accuracy performance effects caused by salt content, all samples should be purified and desalted prior to the analysis with the High Sensitivity DNA kit.

Conclusion

The Agilent High Sensitivity DNA kit complements the existing Agilent 2100 Bioanalyzer DNA kits. It is suitable for highly sensitive quantification and sizing of DNA fragments and smears in the size range of 50 to 7000 bp. Table 1 summarizes the excellent performance of the High Sensitivity DNA kit.

The Agilent High Sensitivity DNA kit used with the Agilent 2100 Bioanalyzer is extremely useful for the analysis of double-stranded DNA samples in next-generation sequencing workflows.

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