Application compendium - 2nd edition

Sample Quality Control in Next-Generation Sequencing Workflows

Agilent Automated Electrophoresis Portfolio

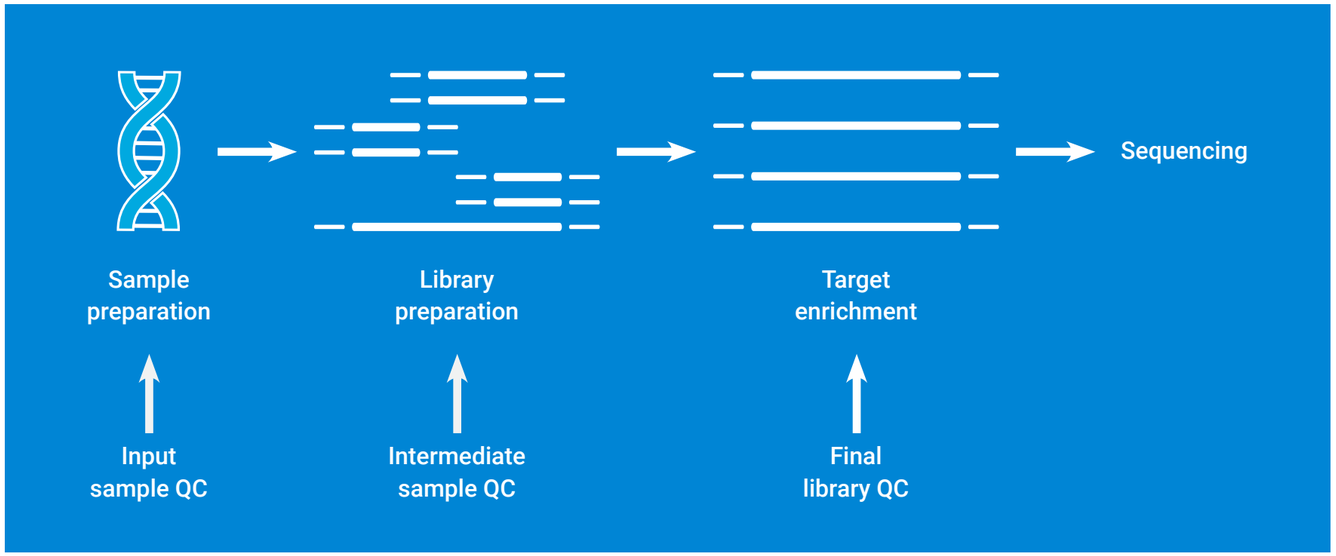
Introduction

Introduction

Throughout the updated edition of this application compendium, you will find various application notes and technical overviews providing you essential information for success in your nucleic acid sample quality assessment during next-generation sequencing (NGS) workflows.

This collection of resources includes topics covering:

- Why sample quality control is important in NGS workflows
- How quality metrics help provide reliable assessment of sample integrity
- How to analyze and assess quality of cell-free DNA (cfDNA), formalin-fixed paraffin-embedded (FFPE) DNA and RNA, genomic DNA (gDNA), total RNA, and NGS library samples
- Which instrument is most suitable for different workflows and applications



Recommended steps for quality control during NGS library preparation.

Automated Electrophoresis Instruments to Support Your NGS Workflows

Next-generation sequencing is an essential tool in molecular biology laboratories for the analysis of nucleic acid samples in numerous basic, translational, and clinical research settings. Quality control (QC) of input samples, at intermediate steps of the preparation process, and of the final libraries before sequencing, can help save time and resources by identifying samples that are of poor quality or of insufficient concentration to yield successful sequencing data.

The Agilent automated electrophoresis portfolio provides several instruments for QC of various nucleic acid sample types to support your NGS workflow.

Discover which Agilent automated electrophoresis solution fits your application. Select an instrument below for more information.

TapeStation systems

Automated electrophoresis QC for various workflows for the analysis of size, quantity, and integrity of RNA and DNA input samples.

Fragment Analyzer systems

Automated parallel capillary electrophoresis providing accurate and reliable quality assessment for different sample types with a broad range of kits.

Femto Pulse system

Automated pulsed-field capillary electrophoresis enabling exceptional QC for low concentration nucleic acid samples and high-molecular weight DNA.

Bioanalyzer system

See how the Agilent Bioanalyzer system compares to our other automated electrophoresis instruments.

Automation for Library Preparation and Target Enrichment

Prepare high-quality, sequencing-ready libraries with more consistency and lab efficiency using automated solutions for library preparation and target enrichment. Choose between two automation platforms from Agilent, both of which support enzymatic fragmentation of DNA, reverse transcription for RNA, and bead cleanup. These automation platforms offer a Reagent Rental Program for SureSelect reagents to offset capital equipment expense.

Bravo NGS system

Simplified NGS workflow automation without sacrificing quality, in a small footprint.

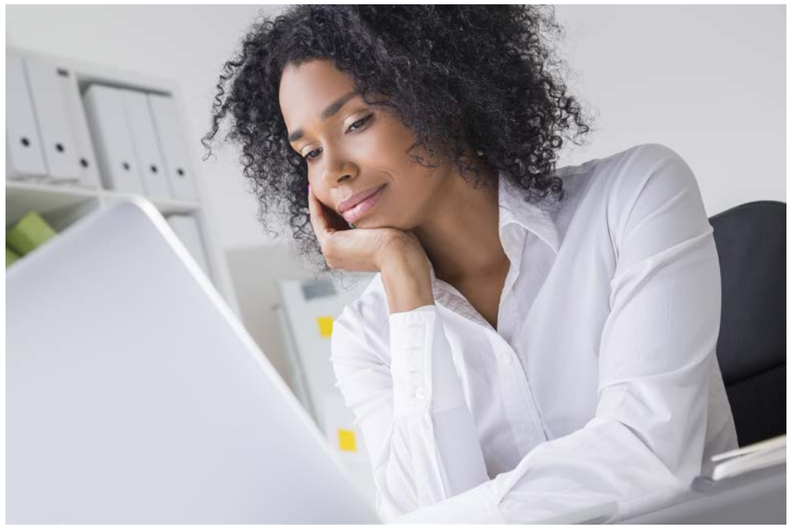
Bravo NGS workstation

High-throughput NGS sample preparation robot for increased efficiency and maximized walkaway time.

Magnis NGS prep system

Compact benchtop platform providing an automated, completely walkaway NGS library preparation and target enrichment solution.

NGS Products For Reliable Results



Besides sample QC instrumentation and automation options, Agilent offers an extensive porfolio of NGS products that help you generate reliable data.

Agilent SureSelect hybridization capture-based reagents include custom and catalog kits for library preparation and target enrichment to meet your sequencing needs. The Agilent Alissa Clinical Informatics platform enables innovative NGS and CGH data analysis to optimize workflows across technologies and applications. The platform seamlessly and efficiently integrates raw read alignment, variant annotation and classification, and reporting modules.

See which products are best suited for your NGS-based research.

Explore the portfolio

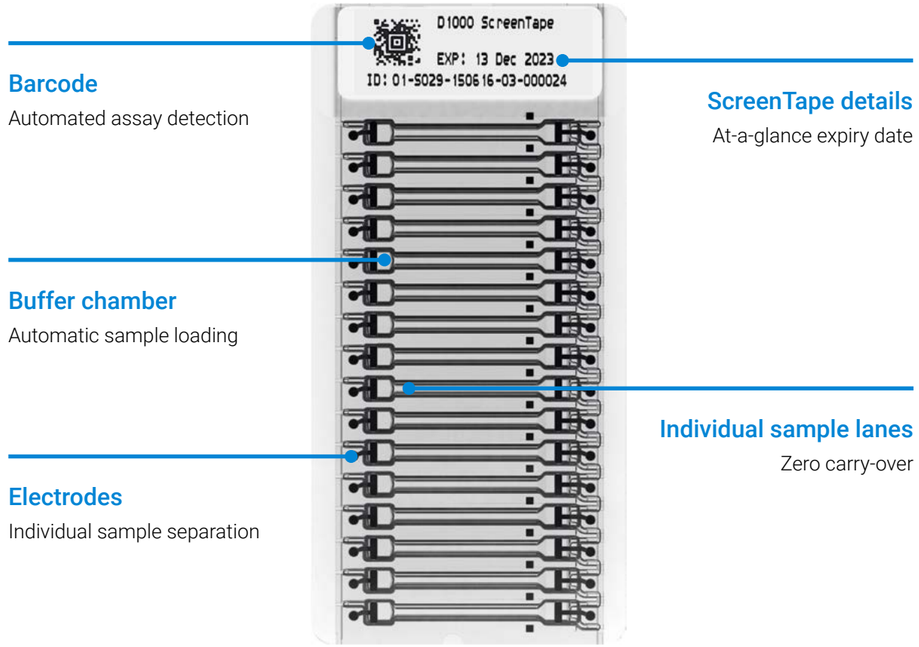
Agilent TapeStation Systems

Key benefits

The Agilent TapeStation systems offer scalable throughput and rapid results, making them the ideal solution for quality control of biological samples in NGS.

While the higher throughput Agilent 4200 TapeStation system can analyze DNA and RNA samples from a 96-well plate, the smaller footprint Agilent 4150 TapeStation instrument is the lower throughput alternative for analyzing up to 16 samples per run. Both systems offer walk away operation with fully automated sample processing.

No matter which system you choose, full assay compatibility is guaranteed as both instruments share the same ScreenTape consumables.



Overview of Agilent ScreenTape device.

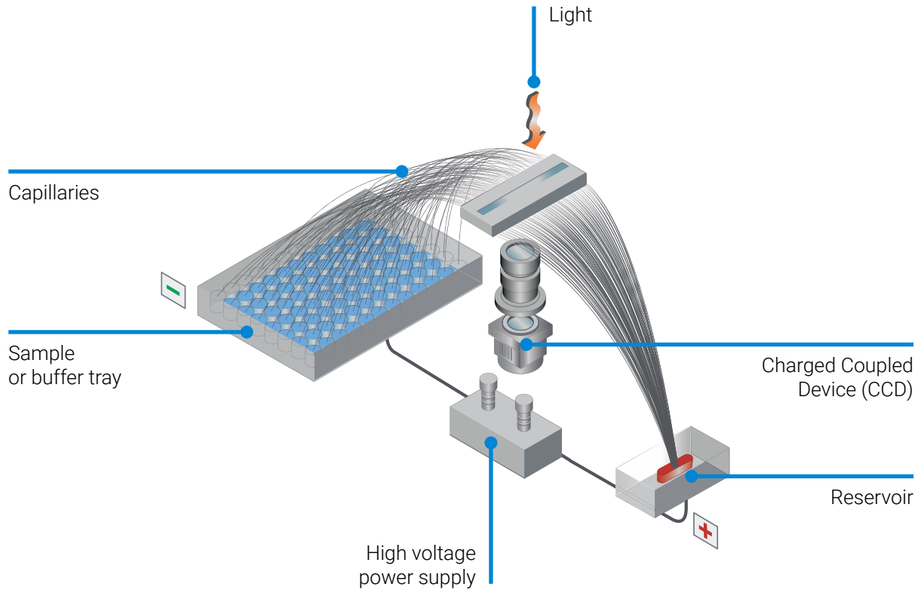
Learn more about the TapeStation systems

Agilent Fragment Analyzer Systems

Key benefits

The Agilent Fragment Analyzer systems use automated parallel capillary electrophoresis (CE) to provide reliable quality control (QC) of nucleic acids for various applications, including next-generation sequencing.

The 5200, 5300, and 5400 Fragment Analyzer systems are used for low to high throughputs, breaking through analytic bottlenecks and streamlining nucleic acid analysis workflows to provide researchers with accurate and reliable results. Automated parallel capillary electrophoresis can analyze multiple samples at once without researcher intervention. The systems can house two different gel matrices enabling unattended and consecutive analysis of multiple RNA and DNA reagent kits.



Overview of capillary electrophoresis process.

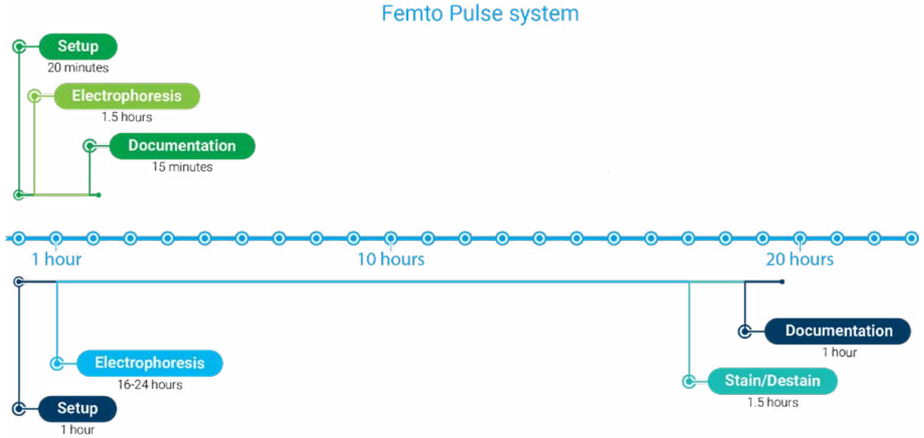
Learn more about the Fragment Analyzer systems

Agilent Femto Pulse System

Key benefits

The Agilent Femto Pulse system delivers unparalleled sensitivity and sizing of nucleic acids, enabling accurate and reliable sizing of gDNA smears and large fragments through 165 kb.

The Femto Pulse system is a powerful and effective automated, pulsed-field capillary electrophoresis system with the ability to separate high-molecular weight (HMW) DNA through 165,000 bp. An optimized optical platform allows the system to easily achieve 10 times higher sensitivity for nucleic acid smears and 100 times higher for nucleic acid fragments compared to the other automated electrophoresis systems. The Femto Pulse was designed for the qualitative and quantitative analysis of nucleic acid sample with low concentrations or HMW DNA.



Overview of capillary electrophoresis process.

Learn more about the Femto Pulse system

Agilent 2100 Bioanalyzer System

For more than **20 years**, molecular biologists have benefited from RNA and DNA analysis using the Agilent 2100 Bioanalyzer system. Starting in January 2024, the Bioanalyzer instrument will no longer be available for purchase, however, its corresponding kits and reagents will remain available.

The new generation of Agilent automated electrophoresis instrumentation, including the TapeStation, Fragment Analyzer and Femto Pulse systems, deliver additional advanced quality metrics for the analysis of specialized sample types such as genomic DNA, cell-free DNA, and total RNA libraries.

To see how sample analysis using the Bioanalyzer system compares to our other automated electrophoresis instruments, explore these resources:

01 Comparison of DNA Sample QC for NGS Workflows with the Agilent Fragment Analyzer and Bioanalyzer Systems
02 Comparison of Small DNA Fragment Analysis using the Agilent Bioanalyzer and Agilent Fragment Analyzer Systems
03 Comparison of Small RNA Analysis using the Agilent Bioanalyzer and Agilent Fragment Analyzer Systems
04 Quality Assessment of NGS Libraries using Agilent Automated Electrophoresis Systems
05 Comparison of the Agilent 2100 Bioanalyzer and the Agilent Fragment Analyzer Systems for Analysis of Plant, Insect, and Bacterial RNA
06 Comparison of DNA Assays Using the 4200 TapeStation System and 2100 Bioanalyzer System
07 Performance Characteristics of the RNA and the High Sensitivity RNA ScreenTape Assays for the 4150 TapeStation System

Proven Quality Metrics for Multiple NGS Applications

Quality metrics provide you with a reliable assessment of sample integrity so you can ensure your nucleic acids are sufficient for successful library preparation and sequencing results. Whether you are working with genomic DNA (gDNA), cell-free DNA (cfDNA), formalin-fixed paraffin-embedded (FFPE) DNA and RNA, or total RNA you can find reliable quality metrics suited to your application.

Explore each of the metrics below and learn about their use in sample quality control with automated electrophoresis instruments.

Bioanalyzer system

Fragment Analyzer systems

Femto Pulse system

TapeStation systems

	Bioanalyzer system	Fragment Analyzer systems	Femto Pulse system	TapeStation systems
Total RNA	+ RIN	+ RQN		+ RIN^e
FFPE RNA	+ DV_{200}			
gDNA/ FFPE DNA		+ GQN		+ DIN
cfDNA				+ %cfDNA

NGS Quality Control for a Wide Range of Nucleic Acids

Robust sample analysis is crucial throughout the NGS workflow for successful sequencing results. Whether you need to assess the integrity of your cell-free DNA (cfDNA), formalin-fixed paraffin-embedded (FFPE) DNA and RNA, genomic DNA (gDNA), total RNA, or NGS libraries, our automated electrophoresis instruments offer you support.

Select your desired sample type to explore relevant application notes and technical overviews:

Genomic DNA analysis +

Total RNA analysis +

FFPE analysis +

Cell-free DNA analysis +

NGS library analysis +

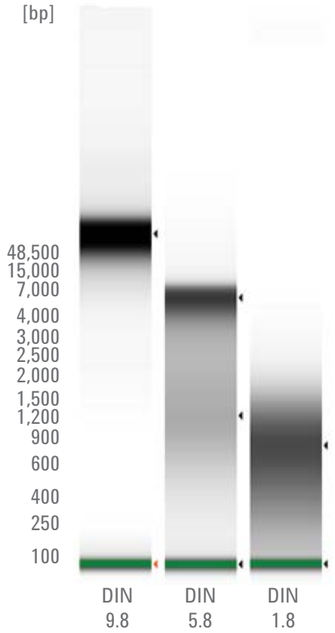
High Throughput Genomic DNA Assessment by the Agilent 4200 TapeStation System

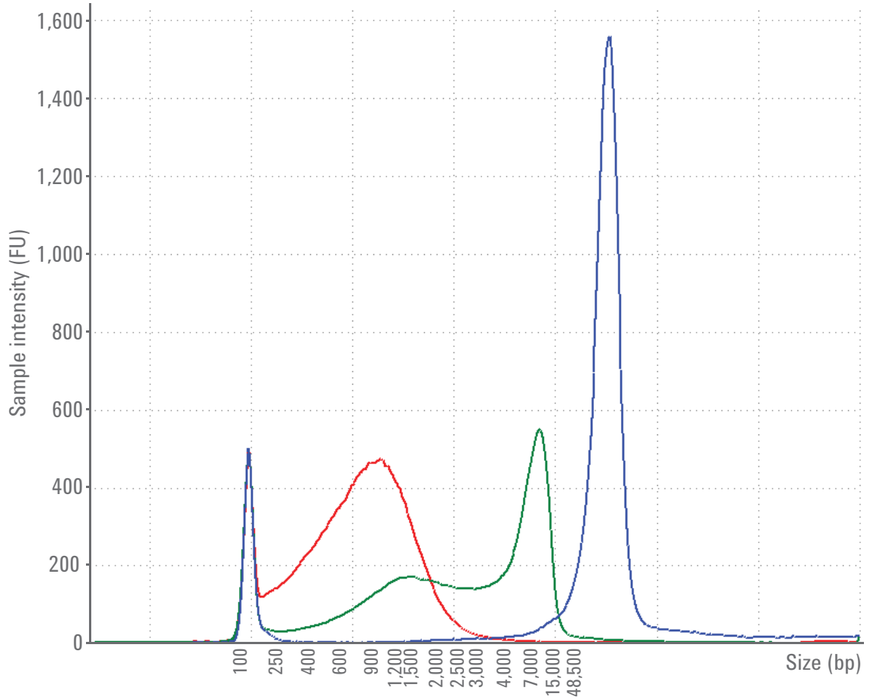
Technical overview

Abstract

When used with the Genomic DNA ScreenTape assay, the 4200 TapeStation system can separate and analyze genomic DNA (gDNA) from 200 to 60,000 bp. It provides an automated numerical assessment of gDNA quality, the DNA integrity number (DIN). The DIN is calculated using a scale from 1 to 10. A high DIN indicates highly intact gDNA, and a low DIN suggests a strongly degraded gDNA sample. The user-independent DIN is the ideal QC tool for next-generation sequencing (NGS) and array comparative genomic hybridization (aCGH) workflows.

Three different mouse gDNA samples with different DNA integrity were analyzed using the 4200 TapeStation system. The TapeStation analysis software displays the results as an electropherogram, a gel image, and data table. The DIN value is automatically determined, and directly displayed under the individual lane of the gel image (A). The corresponding samples are shown in the electropherogram overlay (B).





Read the full technical overview

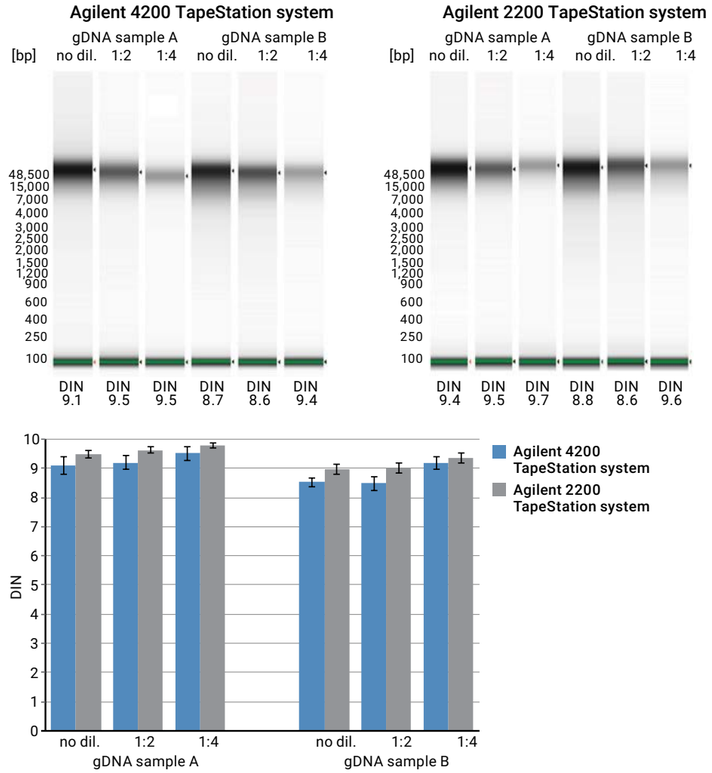
Evaluating the Agilent 4200 TapeStation System for High Throughput Sequencing Quality Control

Application Note

Abstract

To evaluate the difference of the DNA integrity number (DIN) between the 2200 and the 4200 TapeStation systems, two commercially available gDNA samples were analyzed at three different concentrations using the Genomic DNA ScreenTape assay. The DNA integrity number (DIN) is automatically determined and directly displayed below the individual lane of the gel image. A DIN is calculated on a scale from 1 to 10. A high DIN indicates highly intact gDNA, whereas a low DIN corresponds to a strongly degraded gDNA. Both gDNA samples were highly intact, as indicated by the determined DIN, which is displayed below the gel image.

The bar chart illustrates that the DNA integrity analysis with the 4200 TapeStation system is highly comparable to that of the 2200 TapeStation system. The precision for the DNA integrity analysis for both systems is 4% for all tested samples and concentrations.



Read the full application note

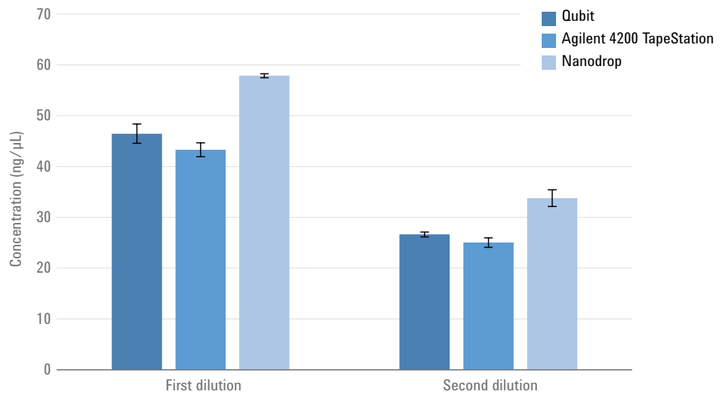
Quality Control for Agilent SureSelect QXT WGS Library Preparation

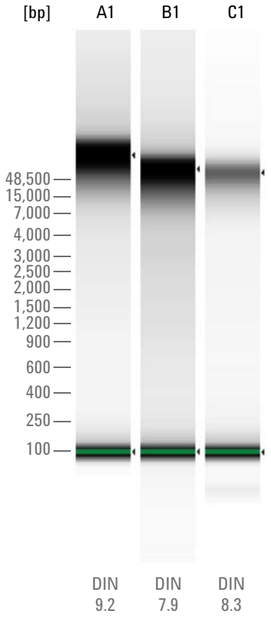
Application note

Abstract

The Agilent SureSelect QXT WGS protocol requires high-quality DNA samples for optimal performance and precise quantification of the gDNA starting material. Serial quantification was carried out using the Qubit instrument and the dsDNA BR assay in accordance with the protocol. The same samples were analyzed on the 4200 TapeStation system with the Genomic DNA ScreenTape assay and the NanoDrop with six replicates on each instrument. Data from the 4200 TapeStation system, Qubit, and NanoDrop are presented in the figure, showing the applicability of the Genomic DNA ScreenTape assay in quantitating genomic DNA starting material. The quantification results of the TapeStation system correlate with the Qubit instrument. The measurement of genomic DNA with UV spectroscopy tends to overestimate the quantity due to other buffer components that may absorb in the UV spectrum.

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





Read the full application note

GQN Quality Metrics with the Fragment Analyzer and Femto Pulse Systems

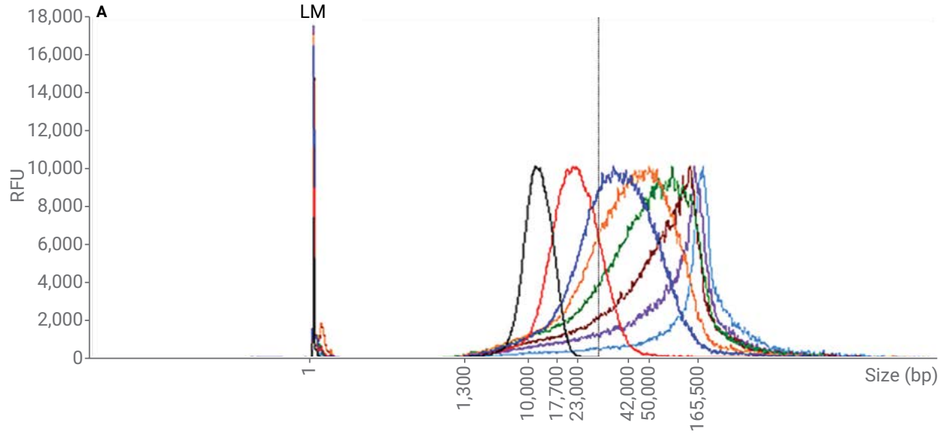
Application note

Abstract

Quality assessment of nucleic acids is critical to the success of many downstream applications, including next-generation sequencing (NGS). The Fragment Analyzer and Femto Pulse systems provide quick and easy assessment of genomic DNA (gDNA) quality and integrity with the genomic quality number (GQN).

The GQN is commonly used for evaluating the input gDNA material for NGS library preparation. To prepare a successful library, the sample must be of the correct size and of sufficient quality for sequencing. The GQN threshold can be set by the user to reflect the size threshold necessary for their particular requirements. The GQN is given on a scale of 0 to 10, with a higher score indicating that more of the sample exceeds the user-defined threshold. In this example, the Femto Pulse system was used to report the average smear size (A) and GQN set at 30 kb (B) of several gDNA samples that had been sheared to various sizes. For NGS, quality metrics can aid decisions about library preparation, including which samples to use for input material, the number of PCR cycles, and the amount of library to use for enrichment.

A



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

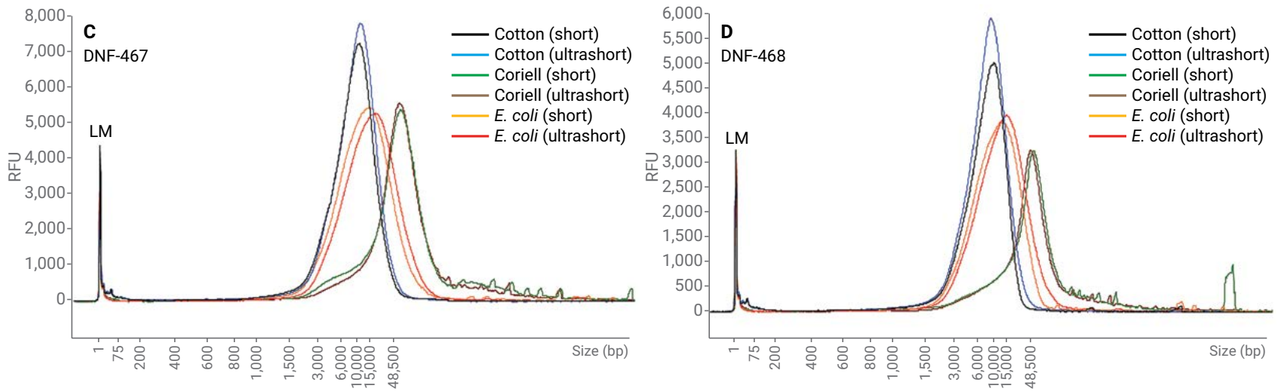
Read the full application note

Assessment of Genomic DNA Quality with the Agilent 5200 Fragment Analyzer System

Application note

Abstract

The quality and concentration of genomic DNA (gDNA) starting material is crucial for successful downstream long-read and whole-genome sequencing. Quality analysis for gDNA with varying ranges of concentrations can be performed on the Fragment Analyzer systems with the Genomic DNA 50 kb kit and the HS Genomic DNA 50 kb kit. The Genomic DNA 50 kb kit offers a concentration range of 25 to 250 ng/µL input gDNA, while the HS Genomic DNA 50 kb kit has a lower concentration range of 0.3 to 12 ng/µL input gDNA for low concentrated samples. Genomic DNA from cotton, *E. coli*, and human (Coriell) were compared on both kits with the FA 12-Capillary Array Short, 33 cm (short array), and FA 12-Capillary Array Ultrashort, 22 cm (ultrashort array). On both kits, the short and ultrashort arrays demonstrated consistent sizing for the three sample types. The ultrashort array offers the convenience of shortened run times while providing comparable gDNA sizing, concentration, and genomic quality number (GQN) compared to the short array with both kits.



Read the full application note

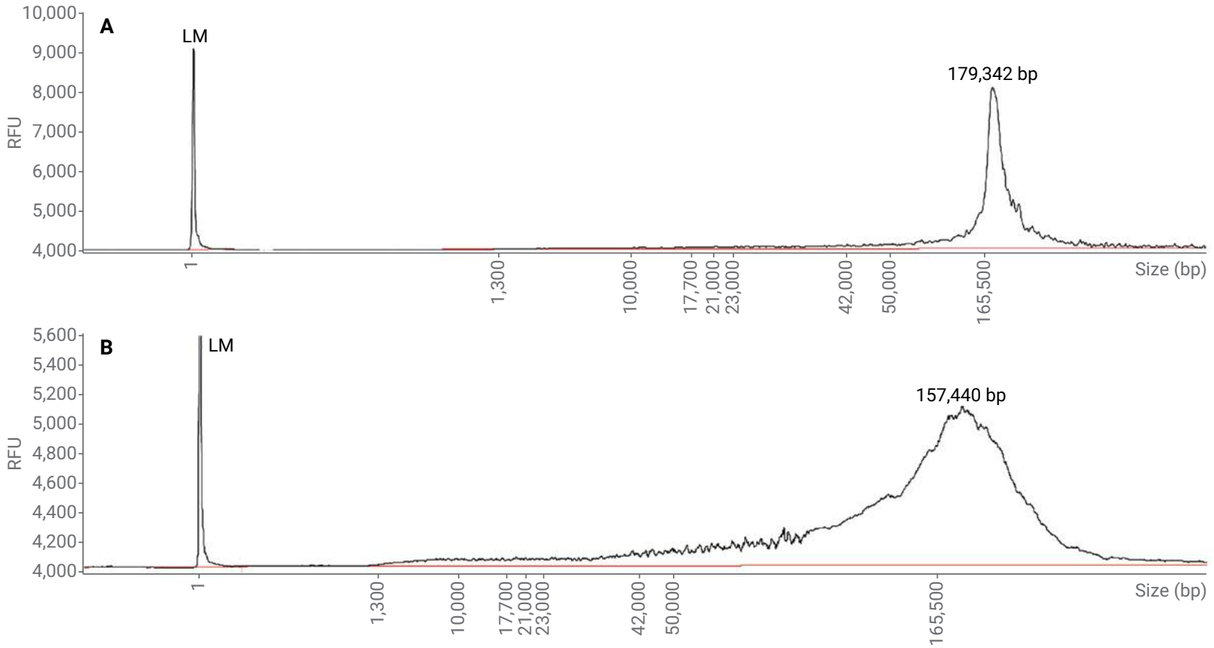
Genomic DNA Sizing and Quality Control on the Agilent Femto Pulse System

Application note

Abstract

Large-insert library preparation relies upon multiple quality control steps. Typically, overnight pulsed-field gel electrophoresis (PFGE) separations are used to assess gDNA over 50 kb in size. The Agilent Femto Pulse system is the only instrument on the market capable of replacing PFGE with fast, automated assessment of high-molecular weight (HMW) gDNA, saving time and money in the preparation of large-insert libraries. The Genomic DNA 165 kb kit offers two pulsed-field capillary electrophoresis separation methods. The gDNA 165 kb method is a 70-minute method recommended for gDNA under 80 kb. The extended Genomic DNA 165 kb method provides enhanced separation and sizing for larger samples in 3.5 hours. Large gDNA separated with the fast method displayed a sharp, compact peak around 165 kb (A). The same sample analyzed with the extended method resulted in a broader smear representing the entire sizing range of the sample (B).

This application note shows comparable sizing of four gDNA samples separated on the Femto Pulse and traditional PFGE. It also demonstrates consistent sizing of samples throughout a dilution series and discusses the application of genomic quality number (GQN) provided by the Femto Pulse system.



Read the full application note

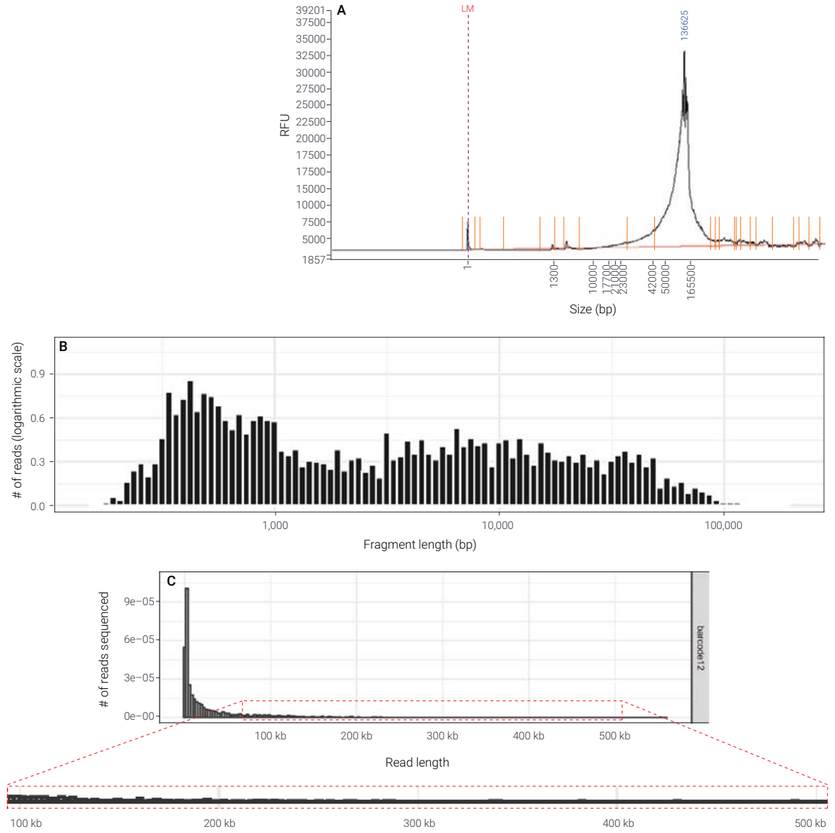
Comparison of Different Methods to Isolate HMW DNA from Bacteria for Nanopore Sequencing

Application note

Abstract

The first step in many sequencing experiments is to isolate nucleic acids from a specimen. As long-read sequencing technologies have advanced, many commercial companies now offer kits specific to the extraction of high-molecular weight (HMW) genomic DNA (gDNA). However, the size and quality of the gDNA can vary greatly depending upon the extraction method used. For sequencing facilities, this can affect the type of sequencing that is performed, making knowledge of sample integrity crucial to a successful experiment.

In this application note, researchers at the NGS Competence Center Tuebingen (NCCT) used the Agilent Femto Pulse system to analyze HMW gDNA from five different commercially available extraction kits. The size of the input material was then compared to the sequenced read lengths to demonstrate the impact that the isolation method can have on sample size and sequencing results. An example from one of the five extraction kits is shown in the figure. Data generated from the Femto Pulse demonstrated that the extraction method used resulted in a large peak with some smearing to the left of the peak, indicating the presence of smaller fragments within the sample (A). The distribution of the sample was confirmed with Nanopore sequencing results, which showed a large amount of fragments of smaller sizes (B), but also some as big as 500 kb (C).



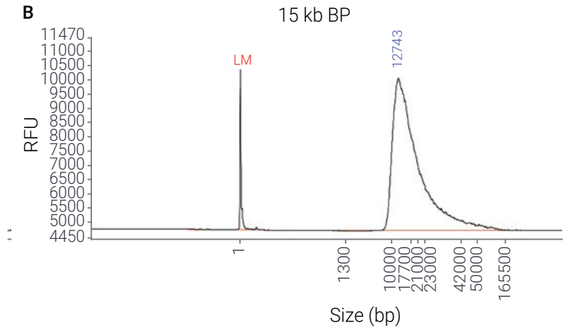
Read the full application note

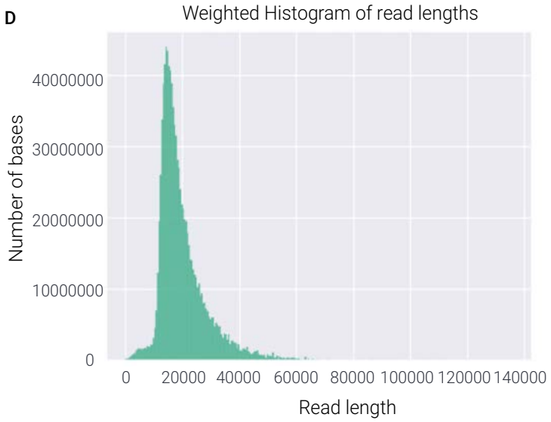
Comparison of Agilent Femto Pulse System Sizing with Long-Read Sequencing Read Length

Application note

Abstract

Long-read sequencing results can be maximized by loading only long fragments onto the sequencer, thereby eliminating any issues with preferential sequencing of smaller fragments. This can be achieved through size selection, to exclude the portion of the sample below a specified threshold. Sheared samples, with and without size selection, were analyzed with the Agilent Femto Pulse system, and then sequenced on the Oxford Nanopore Technologies MinION. The size distribution of the samples reported by the Femto Pulse was similar to the histogram of the sequencing read lengths. Shown here is an example of a 15 kb Blue Pippin (15 kb BP) size-selected gDNA analyzed on the Femto Pulse (B). The sample was sequenced with the Oxford Nanopore Technologies MinION, and histograms of the read lengths were generated from the data with NanoPlot (D). Size selection eliminated the small fragments present in the sample, resulting in similar sizes between the Femto Pulse average size and the mean sequencing results. The Femto Pulse confirmed effective size selection, which was further confirmed by the sequencing results.





Read the full application note

Comparison of Constant- and Pulsed-Field Electrophoresis Technologies for Analysis of High Molecular Weight and Large DNA Fragments

Application note

Abstract

Choosing the appropriate electrophoresis technology is critical to achieving successful downstream results. Constant- and pulsed-field electrophoresis technologies are both designed to separate DNA based on size. Constant-field technology is traditionally thought to be best suited for separation of DNA smears that are less than 20 kb, based on previous studies. Beyond 20 kb, DNA smears that are analyzed using constant-field technology may be impacted by the compression of samples, which occurs due to the stacking of DNA within the gel. Pulsed-field technology is capable of separating DNA smears with no known compression effects, as seen in constant-field technology. Agilent offers two capillary electrophoresis instruments that use these different technologies: the Agilent Fragment Analyzer systems use constant-field technology, while the Agilent Femto Pulse system uses both pulsed- and constant-field technologies. As shown in the figures, the data presented in this application note indicates that while both the Fragment Analyzer and Femto Pulse can be used for analysis, the Femto Pulse provides more accurate sizing for high molecular weight (HMW) DNA, genomic DNA (gDNA), and sheared samples, both within the range of 10 to 20 kb, and above.

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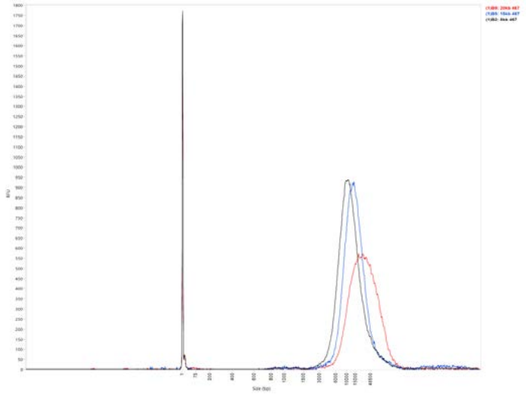

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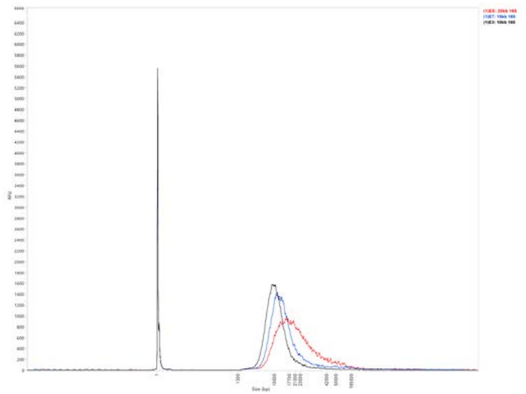

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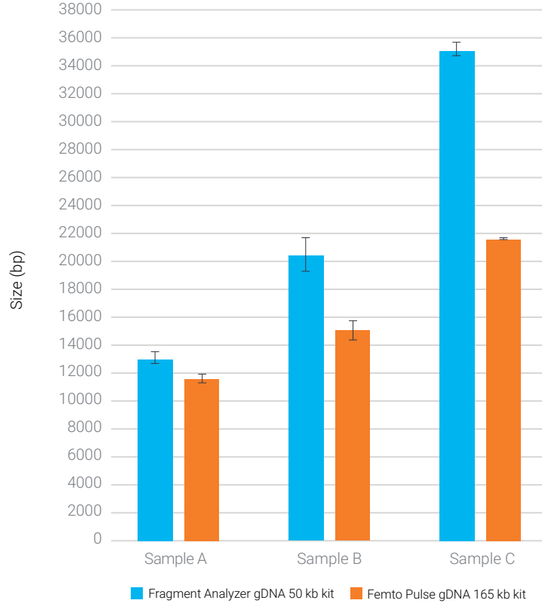


Expected sample size

A) 10 kb

B) 15 kb

C) 20 kb

Read the full application note

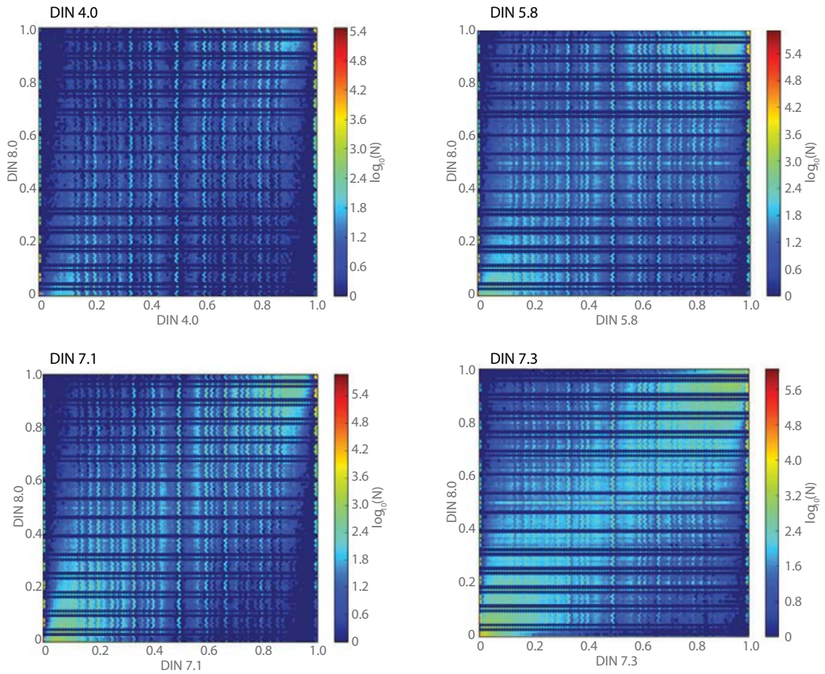
Impact of gDNA Integrity on the Outcome of DNA Methylation Studies

Application note

Abstract

DNA methylation is the most widely studied modification involved in epigenetics. In mammalian cells, DNA methylation mainly involves the transfer of a methyl group from S-adenosyl methionine to the carbon 5 position of a cytosine residue to produce 5-methylcytosine. DNA methylation is mainly implicated in the repression of transcriptional activity.

This application note focuses on 5-methylcytosine (5-mC) DNA methylation and its detection. By far, the most commonly used method for DNA methylation analysis is bisulfite sequencing due to its high-resolution detection when combined with sequencing. Advances in next-generation sequencing made it possible to perform bisulfite sequencing at a genome-wide scale. The DNA methylation analysis based on reduced representation bisulfite sequencing, a high-throughput technique, allows analysis on a single nucleotide level. This method is based on the enrichment of genome regions with high CpG content (sites within the genome where a cytosine is next to a guanine) using a combination of restriction enzymes and bisulfite sequencing and is applicable to any species with a reference genome. Shown here is a comparison of the DNA methylation levels for overlapping CgP sites of four FFPE samples. DIN values ranged from 4 to 7.3, to a fresh frozen sample with a DIN value of 8 using Hexbin plots.



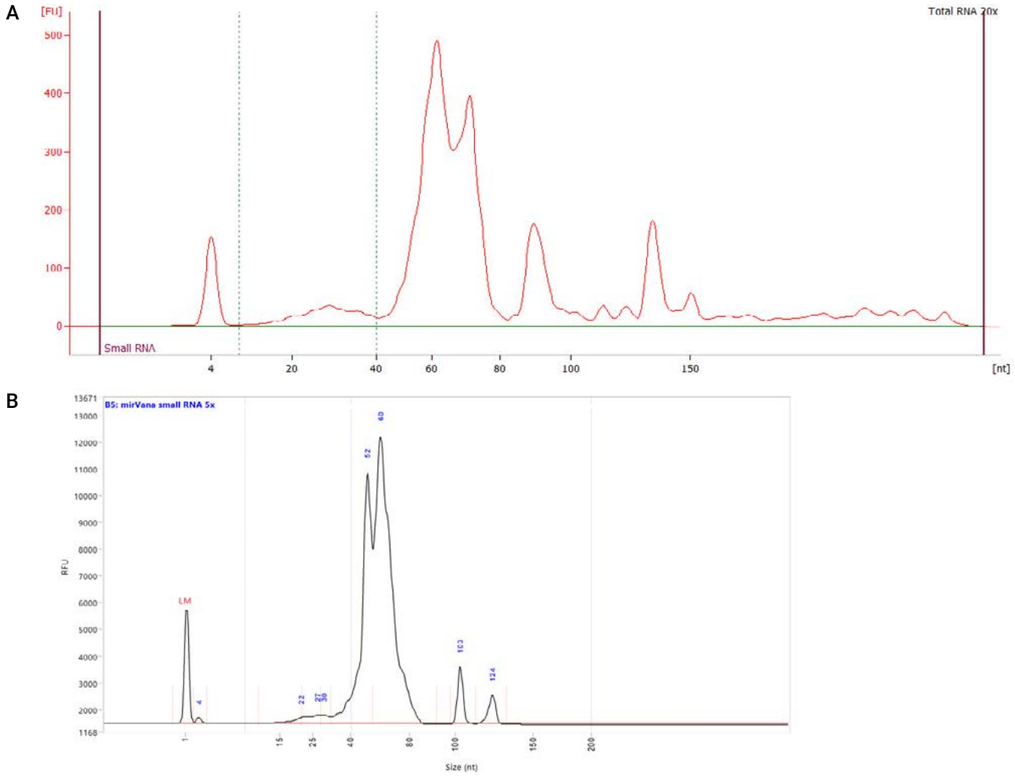
Read the full application note

Comparison of Small RNA Analysis using the Agilent Bioanalyzer and Agilent Fragment Analyzer

Technical overview

Abstract

Analysis of small RNA samples can help with optimization of small RNA isolation and purification protocols and ensure successful downstream applications such as small RNA next-generation sequencing (NGS) (RNA-Seq), miRNA microarrays, and qRT-PCR. The systems of the automated electrophoresis portfolio from Agilent have been well established for the analysis of total RNA, and uniquely offer quality analysis of small RNAs with either the Agilent 2100 Bioanalyzer system, or the Agilent Fragment Analyzer systems. Both instruments use kits that focus on small RNA and miRNA quality control, concentrating on a narrow range of 200 nt and below, and allowing for high-separation resolution of small RNAs. In this technical overview, samples are compared across both the Bioanalyzer and the Fragment Analyzer Small RNA kits to demonstrate the equivalency of the systems for analyzing small RNAs.The figure shows a commercially available total RNA that was further analyzed on A) the Agilent Bioanalyzer Small RNA kit and B) the Agilent Fragment Analyzer Small RNA kit to allow for higher separation resolution of the small RNA portion of the sample below 200 nt.



Read the full technical overview

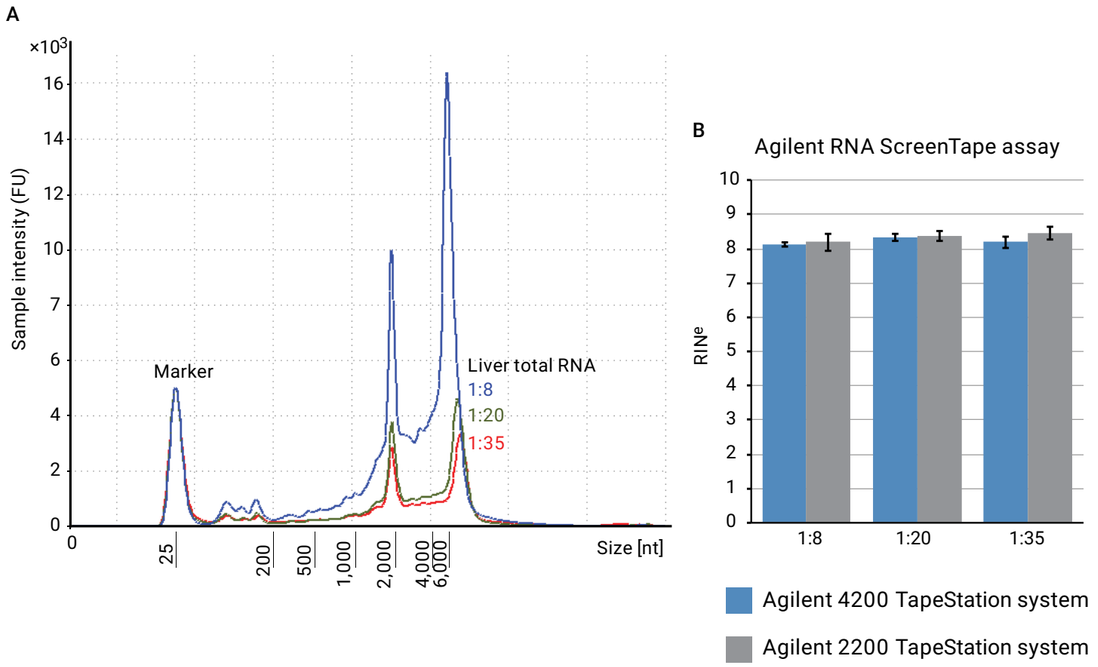
Evaluating the Agilent 4200 TapeStation System for High Throughput Sequencing Quality Control

Application note

Abstract

As RNA is highly sensitive to degradation, quality control is essential, and usually includes RNA integrity analysis and quantification. The electropherogram overlay shown here represents different dilutions of liver total RNA analyzed on the 4200 TapeStation system with the RNA ScreenTape assay (A).

The RNA integrity number equivalent (RIN^e), indicating RNA integrity, is automatically determined by the TapeStation software. The RIN^e is calculated on a scale from 1 to 10. A high RIN^e indicates highly intact total RNA, whereas a low RIN^e corresponds to a strongly degraded sample. The bar chart (B) illustrates that RNA integrity analysis is highly reproducible and independent of the analyzed concentration for both TapeStation systems. In comparison to the 2200 TapeStation system, the 4200 TapeStation system further reduces manual operating time, enabling the analysis of 96 samples without physical intervention. This is a primary requirement for high-throughput NGS labs.



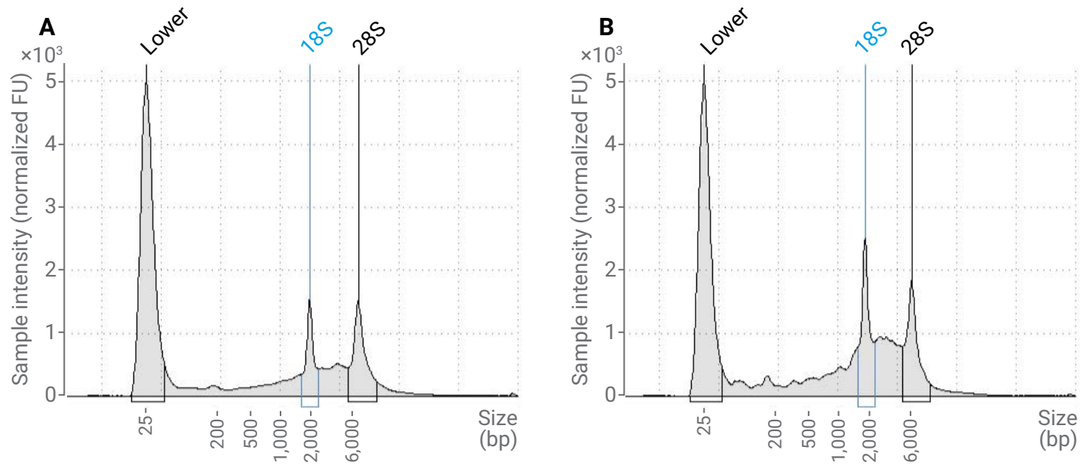
Read the full application note

Quality Control in Illumina Sequencing Workflows Using the TapeStation System

Application note

Abstract

RNA is sensitive to degradation due to the ubiquitous presence of RNase and its more fragile single-stranded structure. Therefore, monitoring the integrity of starting material is indispensable, and processing a reference sample as positive control throughout the library preparation and sequencing is highly advisable. With the RNA ScreenTape assay, the RNA integrity number equivalent (RIN^e) delivers an objective assessment of the integrity of RNA starting material. RIN^e is a proven equivalent to the widely accepted quality metric RIN. The fragmentation conditions of RNA-seq protocols used by the sequencing core facility are optimized for high-quality RNA; more precisely, a RIN^e of 8.0 or higher is recommended for successful library preparation. The figure shows two samples close to this threshold, one passing (A) and one failing (B) the quality requirement. The use of degraded RNA can result in low yield, over-representation of 3' ends of the RNA molecules, or failure of the protocol.



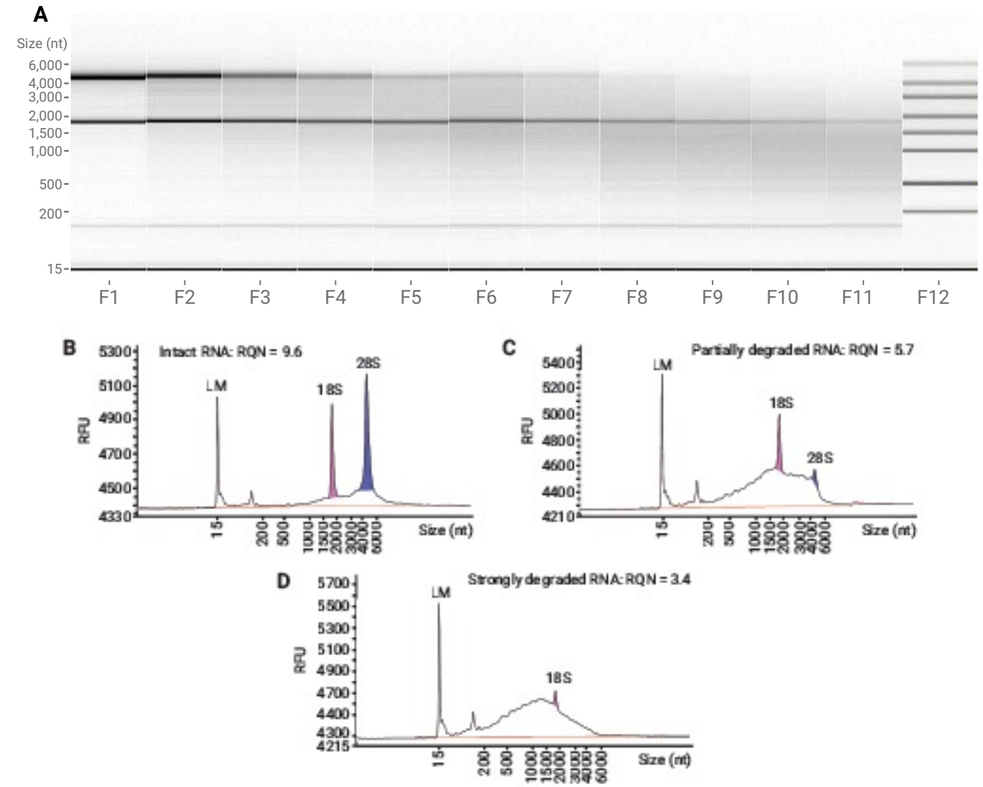
Read the full application note

Quality Analysis of Eukaryotic Total RNA with the Agilent 5200 Fragment Analyzer System

Application note

Abstract

The RNA quality number (RQN) is a user-independent quality metric for easy evaluation of total RNA quality. Total RNA quality is a constant concern because of how easily RNA degrades due to heat, RNase exposure, and improper handling. The 5200 Fragment Analyzer system and RQN metric were used to analyze universal mouse reference total RNA degradation over time. Electropherogram (A) and digital gel images (B) allow for the total RNA profiles to be compared at different time points of heat degradation. As shown, the 18S ribosomal peak becomes smaller, while the 28S ribosomal peak completely disappears as the sample is further degraded. This degradation strongly correlates with a decrease in RQN (C), allowing for easy assessment of RNA quality.



Read the full application note

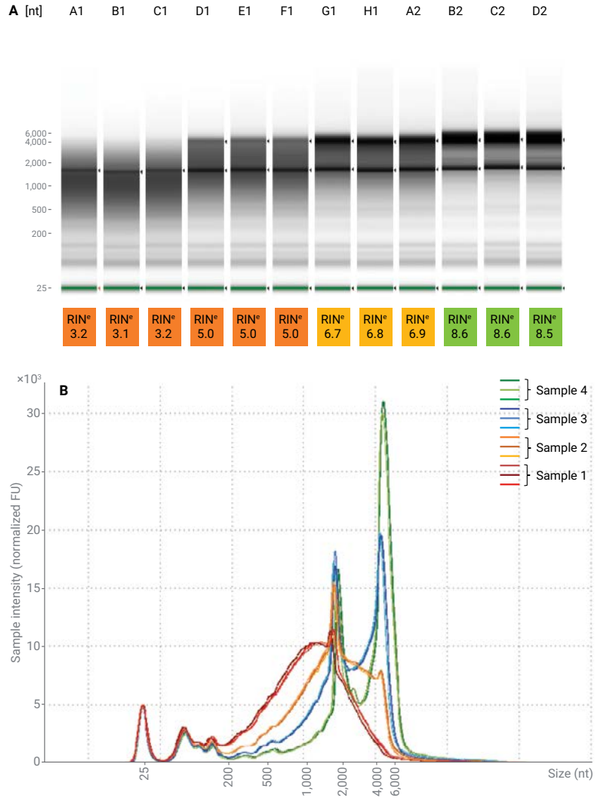
Performance Characteristics of the RNA and High Sensitivity RNA ScreenTape Assays for the 4150 TapeStation System

Technical overview

Abstract

RNA serves as input material within various gene expression analysis techniques like RNA-Seq, microarray, and RT-qPCR. RNA is very sensitive to degradation and its integrity critically affects the success of the downstream applications. Quality control of RNA input material is crucial for ensuring high-quality results. The 4150 TapeStation system can be used with the RNA ScreenTape assay to separate and analyze RNA, providing an automated numerical assessment of RNA quality, the RNA integrity number equivalent (RIN^e). The RIN^e is calculated using a scale from 1 to 10. A high RIN^e indicates highly intact RNA, and a low RIN^e suggests a strongly degraded RNA sample. The RIN^e was demonstrated to be equivalent to RIN from the 2100 Bioanalyzer system. The user-independent RIN^e is the ideal QC tool for next-generation sequencing (NGS) workflows.

Four rat kidney RNA samples with different degradation stages were analyzed using the 4150 TapeStation system. The 4150 TapeStation software displays the results as an electropherogram, a gel image, and data table. The RIN^e value is automatically determined, and directly displayed under the individual lane of the gel image (A). (B) shows the corresponding electropherogram overlay. Comparison of the RIN^e from the 4150 and 4200 TapeStation systems was evaluated and found to be equivalent.



Read the full technical overview

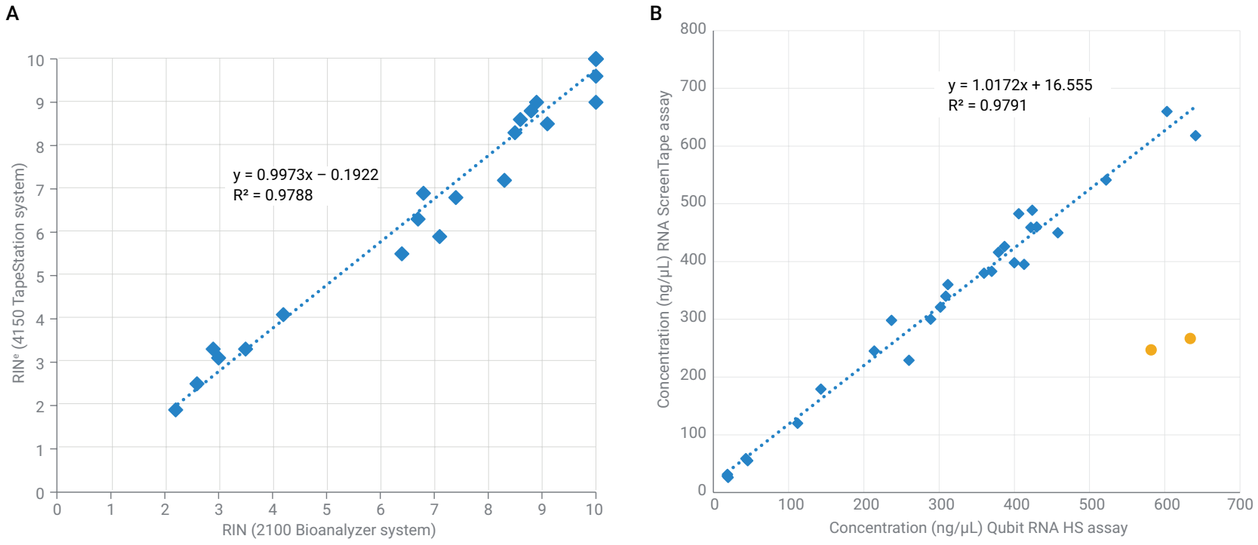
Monitoring Library Preparation for Next-Generation Sequencing in Systems Biology Omics Analysis

Application note

Abstract

RNA samples are often subject to degradation by RNases, chemical, or other environmental impacts. Therefore, RNA integrity analysis is a crucial step before any downstream application like NGS. The RIN^e metric from the TapeStation software is equivalent to the RIN metric provided by the RNA assays of the Bioanalyzer system. The comparative analysis of 30 RNA samples extracted from mouse cancer tissue verified that RIN and RIN^e highly correlate, with a slope of 0.997 and a goodness-of-fit (R^2) of 97.9% (A).

The same samples were quantified fluorometrically and results were compared with the RNA ScreenTape assay. Both assays resulted in a similar total RNA concentration of samples, with a slope of 1.017 and a R^2 of 97.9%, showing high correlation (B). Two degraded RNA samples were treated as outliers. Overall, the RNA ScreenTape assay provides a very useful tool in determining integrity and quantity of RNA starting material for sequencing in a single analysis step.



Read the full application note

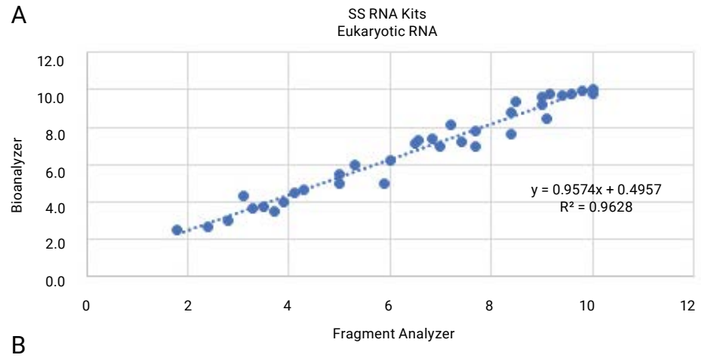
Comparison of RIN and RQN for the Agilent 2100 Bioanalyzer and the Fragment Analyzer Systems

Technical overview

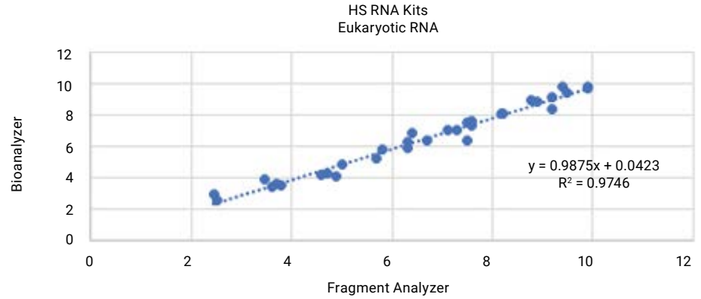
Abstract

The Bioanalyzer instrument is well established for providing a reliable, automated RNA integrity number (RIN). The RIN provides an objective assessment of RNA integrity. The Fragment Analyzer offers a user-independent quality metric, the RNA quality number (RQN), for easy evaluation of total RNA quality. Both the RIN and RQN consider the entire electropherogram, with scoring from 1 to 10, where 10 indicates the highest possible RNA quality and 1 completely degraded RNA. Eukaryotic samples with a varying degree of RNA integrity, from completely intact, to mildly and strongly degraded, were compared on the Bioanalyzer and Fragment Analyzer instruments. Both the standard sensitivity (A) and High Sensitivity RNA (B) kits on both instruments provided comparable RIN and RQN scores throughout the degradation series. This is demonstrated with the slope and R^2 value close to 1. This technical overview also shows a strong correlation between the RIN and RQN for prokaryotic *E. coli* RNA samples.

A



B



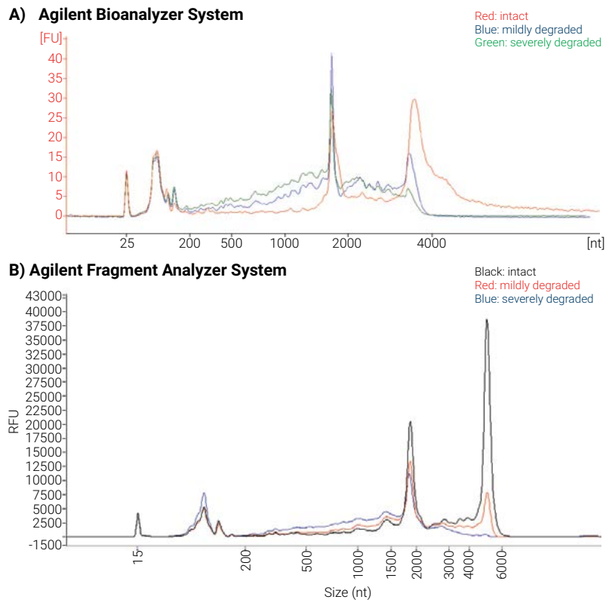
Read the full technical overview

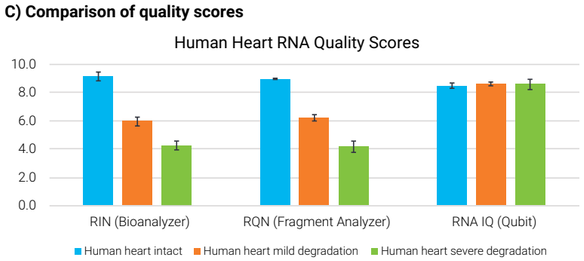
Comparison of RNA Quality Analysis with the Qubit RNA IQ Assay and Agilent Automated Electrophoresis Systems

Technical overview

Abstract

RNA integrity can be determined using electrophoresis methods that separate the sample based on size, allowing for the distinction of the ribosomal peaks, small RNA, and any degradation products. The Agilent 2100 Bioanalyzer system and the Agilent Fragment Analyzer systems are used for RNA quality control (QC), providing information about both the quantity and the quality of a given sample. The systems both generate an objective, reliable quality metric score for each sample analyzed. These scores are known as the RNA integrity number (RIN) for the Bioanalyzer, and the RNA quality number (RQN) for the Fragment Analyzers. Each score is based on a scale from 1 to 10, with 1 identifying significantly degraded samples and 10 identifying high-quality, intact RNA specimens. The RNA Integrity and Quality (IQ) Assay kit for the Qubit 4.0 fluorometer from Thermo Fisher Scientific also offers an RNA quality score, representative of the ratio of small and large RNAs present in the sample. In this technical overview, the RIN and RQN scores from the Agilent automated electrophoresis instruments are compared to the Qubit RNA IQ score across a series of RNA reference samples from multiple species.





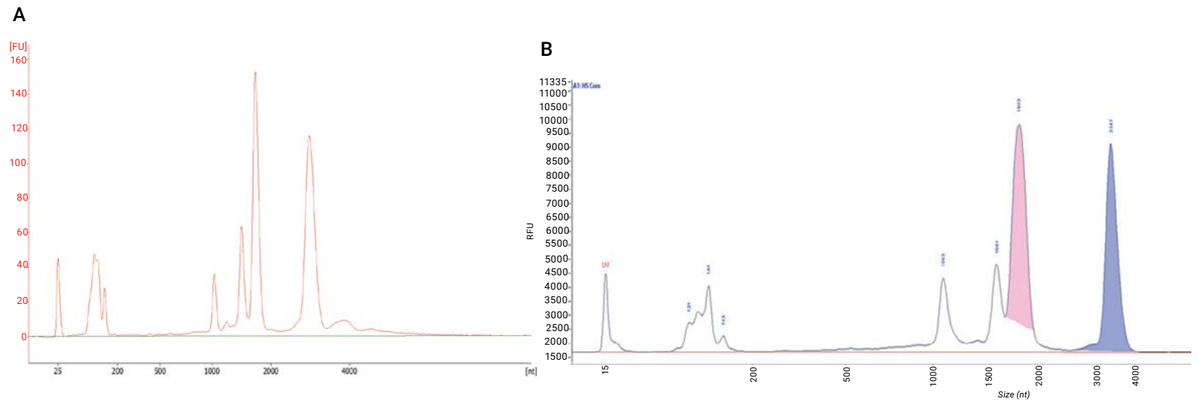
Read the full technical overview

Comparison of the Agilent 2100 Bioanalyzer and the Agilent Fragment Analyzer Systems for Analysis of Plant, Insect, and Bacterial RNA

Technical overview

Abstract

Evaluating RNA integrity prior to downstream analysis saves time, effort, and resources by ensuring that only samples of sufficient quality are used. To aid in objectively determining the integrity of a sample, quality metrics can be assigned that help to grade the quality of the RNA, independent of user bias. The Agilent 2100 Bioanalyzer and 5200 Fragment Analyzer systems each provide reliable, convenient analysis of samples. Each system also provides a quality metric for RNA samples, called the RNA integrity number (RIN) and the RNA quality number (RQN), respectively. The RIN and RQN assign a score from 1 to 10 for a given RNA sample. A score of 1 indicates severely degraded RNA, while a score of 10 indicates highly intact RNA. In this technical overview, different total RNA samples were evaluated to compare the quality metrics between the Bioanalyzer and Fragment Analyzer systems. Plant, insect, and bacterial total RNA samples were analyzed using both the high- and standard sensitivity kits for each system, to provide a detailed comparison of the RIN and RQN. The electropherograms here show corn analyzed on the (A) Agilent 2100 Bioanalyzer system and (B) Agilent 5200 Fragment Analyzer system.



Read the full technical overview

Use of the Agilent 4200 TapeStation System for Sample Quality Control in the Whole Exome Sequencing Workflow at the German Cancer Research Center (DKFZ)

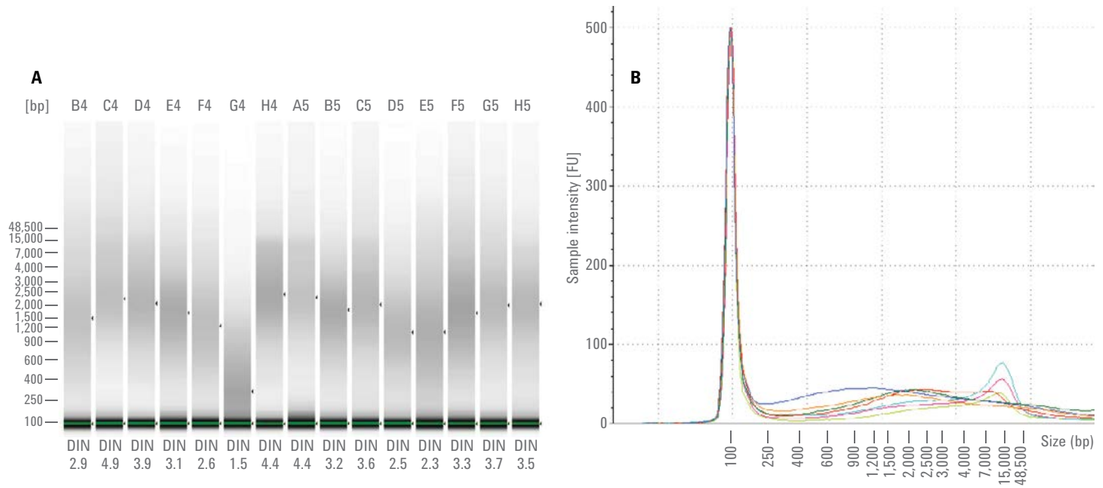
Application note

Abstract

To determine if samples were suitable for NGS library preparation, a quality control (QC) assessment was performed at the beginning for a batch of 88 genomic DNA (gDNA) samples from FFPE tumor tissue by the German Cancer Research Center (DKFZ) High Throughput Sequencing Unit.

This initial QC includes quantification and analysis with a 4200 TapeStation system and the Genomic DNA assay to determine DNA quality, based on the DNA integrity number (DIN).

Both figures show a representative subset of samples analyzed on the 4200 TapeStation with the Genomic DNA ScreenTape assay. gDNA samples extracted from FPPE material often have low DNA integrity but can still be sufficiently intact for whole-exome sequencing library preparation protocols and successful sequencing.



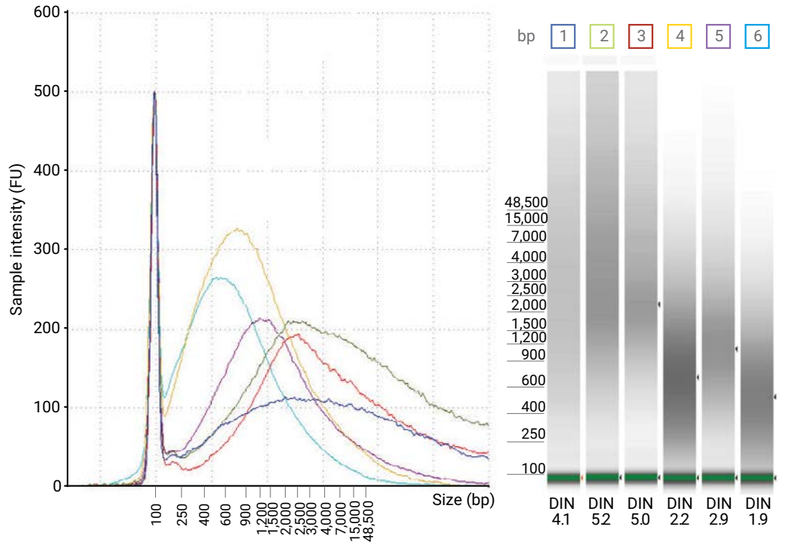
Read the full application note

The DNA Integrity Number (DIN) Provided by the Genomic DNA ScreenTape Assay Allows for Streamlining of NGS on FFPE Tissue Samples

Application note

Abstract

Sequencing of genomic DNA (gDNA) from FFPE archived tissue can be challenging, as the obtained material is often of variable quality. This study demonstrates that the DNA integrity number (DIN) obtained by the quality control of gDNA using the Agilent Genomic DNA ScreenTape assay has allowed for a pronounced saving of sequencing and sample preparation overhead. Out of a total of 751 FFPE samples, a subset of 197 were tested for a correlation of various NGS parameters against the DIN. A correlation was identified between DIN and the key parameters of on-target rate and coverage at 10x. A QC threshold of ≥ 3 DIN was therefore set, which consequently excluded 65% (n = 488) of the total sample set and saved a significant amount of time and effort. The right panel is the gel image, and the left panel is the electropherogram.



Read the full application note

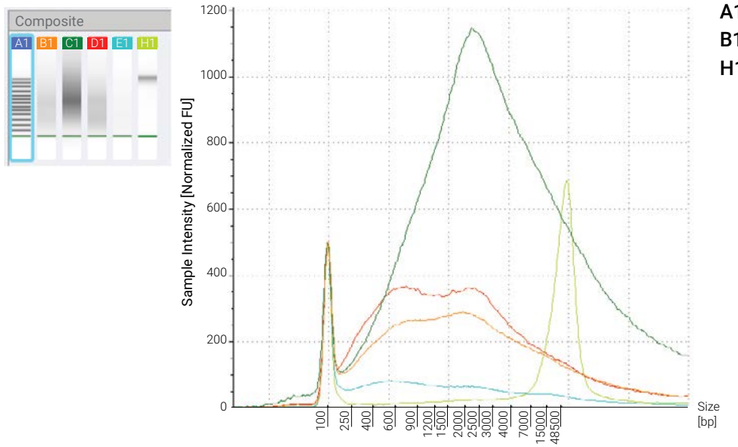
FFPE Sample Quality for the MGISEQ-2000 Sequencing Platform with the Agilent TapeStation System

Application note

Abstract

Formalin-fixed paraffin embedding (FFPE) is one of the more common ways to preserve clinical samples, and FFPE tumor samples serve as valuable study materials for clinical and translational medicine research. To ensure successful and reliable sequencing of FFPE-derived DNA, it is necessary to conduct quality control (QC) of both the initial DNA, as shown here, as well as fragmented samples at key points during next-generation sequencing (NGS) library construction. In this application note, the Agilent 4150 TapeStation system was used to conduct QC of the entire library construction process for five FFPE DNA samples used on the MGI Tech MGISEQ-2000RS sequencing platform. High-quality sequencing results were achieved by stepwise QC throughout the library preparation process, including the initial sample, pre- and post-PCR steps, and the final library, helping to ensure high-quality sequencing.

No.	Type of Tissue	DIN	Concentration (ng/µL)	Main Peak (bp)
FFPE-1	Cervical	2.9	28.6	1,919
FFPE-2	Colorectal	4.5	84.8	2,241
FFPE-3	Thyroid	2.6	42.4	731
FFPE-4	Breast	2.2	10.2	574
Control	Standards	7.9	32.4	24,312



A1) gDNA kit ladder (100 bp - 48.5 kb)
B1 to E1) FFPE samples 1 to 4
H1) FFPE DNA standard

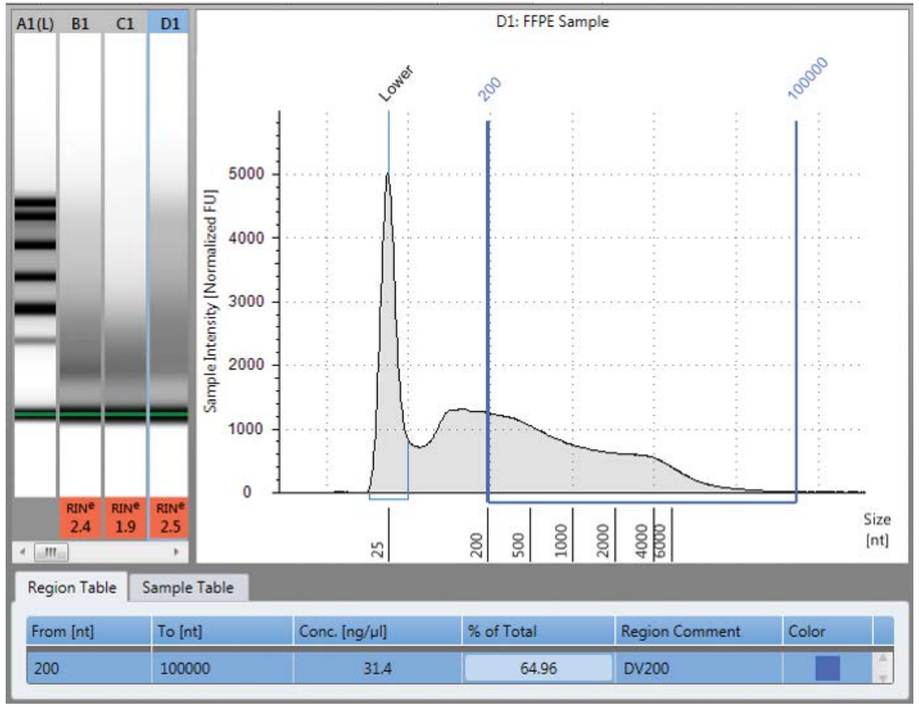
Read the full application note

DV_{200} Evaluation with RNA ScreenTape Assays

Technical overview

Abstract

The DV_{200} quality metric represents the percentage of RNA fragments above 200 nucleotides and shows a high correlation to the precapture library yield of RNA samples originating from formalin-fixed paraffin-embedded (FFPE) tissue. The RNA and High Sensitivity RNA ScreenTape assays enable fast and easy analysis of FFPE RNA samples, with the TapeStation software displaying DV_{200} results after region setup as percentage of total. DV_{200} region setup can be automated for repeated FFPE RNA sample analysis and all region data can be exported and reported. Both the RNA ScreenTape assay and High Sensitivity RNA ScreenTape assay yielded highly comparable results.



Read the full technical overview

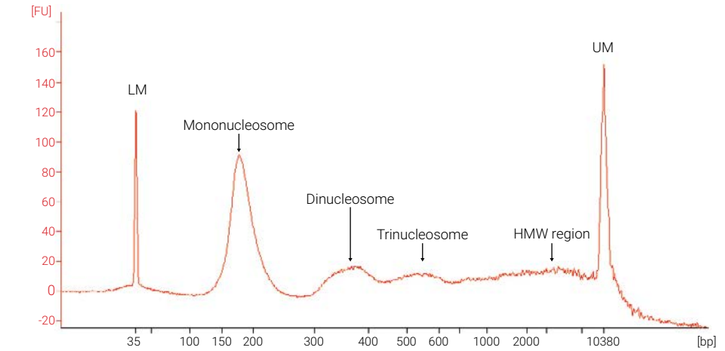
Detection of Contaminating High Molecular Weight DNA with the Cell-Free DNA ScreenTape Assay

Technical overview

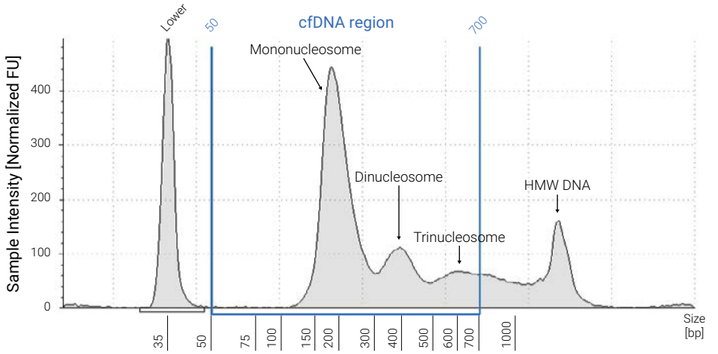
Abstract

Cell-free DNA represents a challenge for next-generation sequencing (NGS) workflows due to the low yield, complex fragmentation pattern, and possibility of contaminating high molecular weight (HMW) DNA. To appropriately assess the quality of cfDNA, it is important to visualize the fragmentation pattern and any degradation or contamination within the sample. Assessment of the cfDNA fragments can be performed using Agilent automated electrophoresis instruments, including the Bioanalyzer and TapeStation systems, as shown in the figure. However, the presence of HMW DNA contamination, which can lead to misrepresentation of the total sample concentration and negatively affect NGS library yield and sequencing results, can overlap with the Upper Marker used by the Bioanalyzer, impacting assessment of the total sample. Alternately, the Cell-free DNA ScreenTape assay for the TapeStation systems reliably visualizes HMW DNA contamination and calculates a %cfDNA quality score for objective assessment of the amount of cfDNA in the sample compared to any HMW DNA contamination. This technical overview compares the analysis of cfDNA contaminated with HMW DNA between the Bioanalyzer and TapeStation systems.

A. Agilent 2100 Bioanalyzer system



B. Agilent 4200 TapeStation system



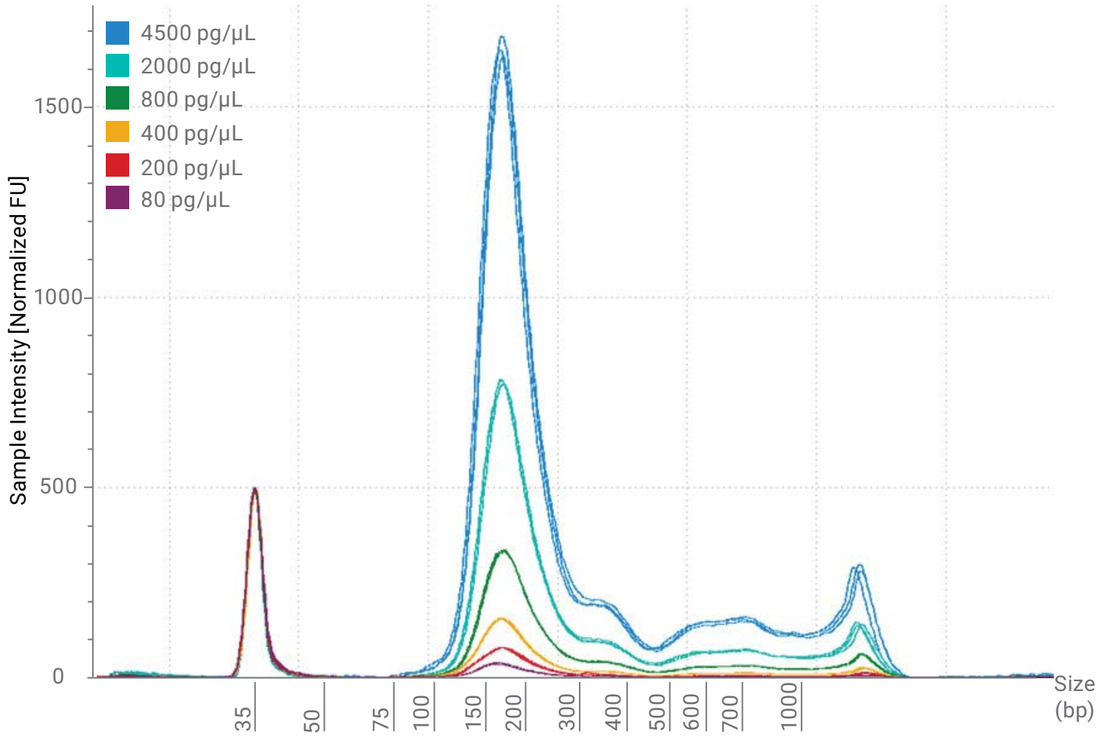
Read the full technical overview

Performance Characteristics of the Agilent Cell-free DNA ScreenTape Assay

Technical overview

Abstract

To demonstrate the concentration independence of the %cfDNA metric, a dilution series of a reference cfDNA sample (n=24), covering the entire concentration range of the assay, was analyzed. The average %cfDNA value was 85.0% ± 1.0 with a minimum value of 83.7% and maximum value of 86.6%. The %cfDNA results and the electropherogram profiles were consistent over the entire concentration range of the assay. In the figure, each concentration is overlaid with three replicates to demonstrate the consistency and precision of the Cell-free DNA ScreenTape assay. These results show that the %cfDNA quality metric provided by the Cell-free DNA ScreenTape assay is highly accurate and precise, and that the percentage is independent of the sample concentration.



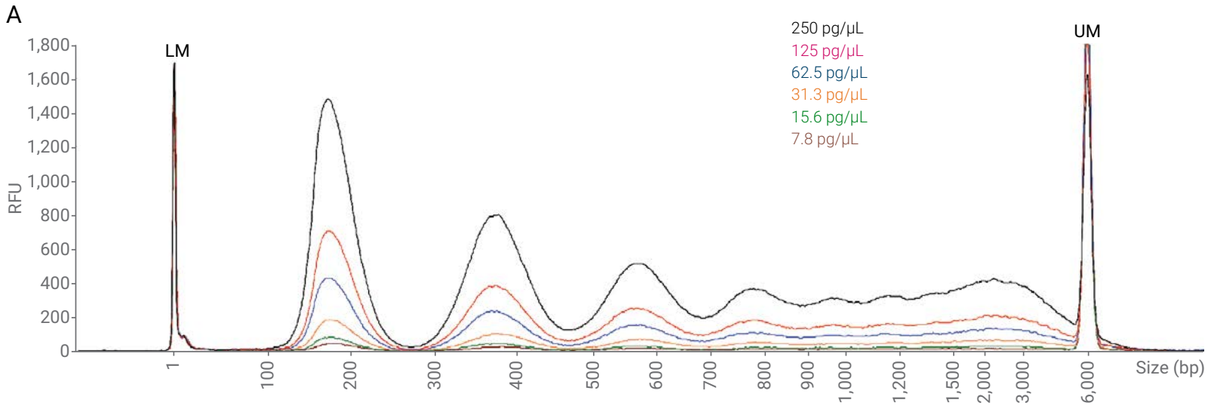
Read the full technical overview

cfDNA Separated on the Agilent Femto Pulse System

Application note

Abstract

The Femto Pulse system offers unparalleled sensitivity for the analysis of low concentrated cfDNA samples. In addition, the system's high resolution provided allows samples with multiple fragments to have complete separation between peaks. cfDNA samples can differ in the number of fragments present, but the mono- and dinucleosome fragments are usually both present. cfDNA analyzed on the Femto Pulse with the Ultra Sensitivity NGS kit displayed six fragments (A). In the dilution series (250 to 7.8 pg/µL), the first three cfDNA fragments were completely separated at all concentrations, with the fourth fragment easily distinguishable down to 15.6 pg/µL. The fifth and sixth fragment peaks were less apparent below 31.3 pg/µL. A seventh fragment peak was observed only in the highly concentrated samples, 62.5 pg/µL and higher. Sizing remained consistent for all six peaks throughout the concentration range they were visible for (B).



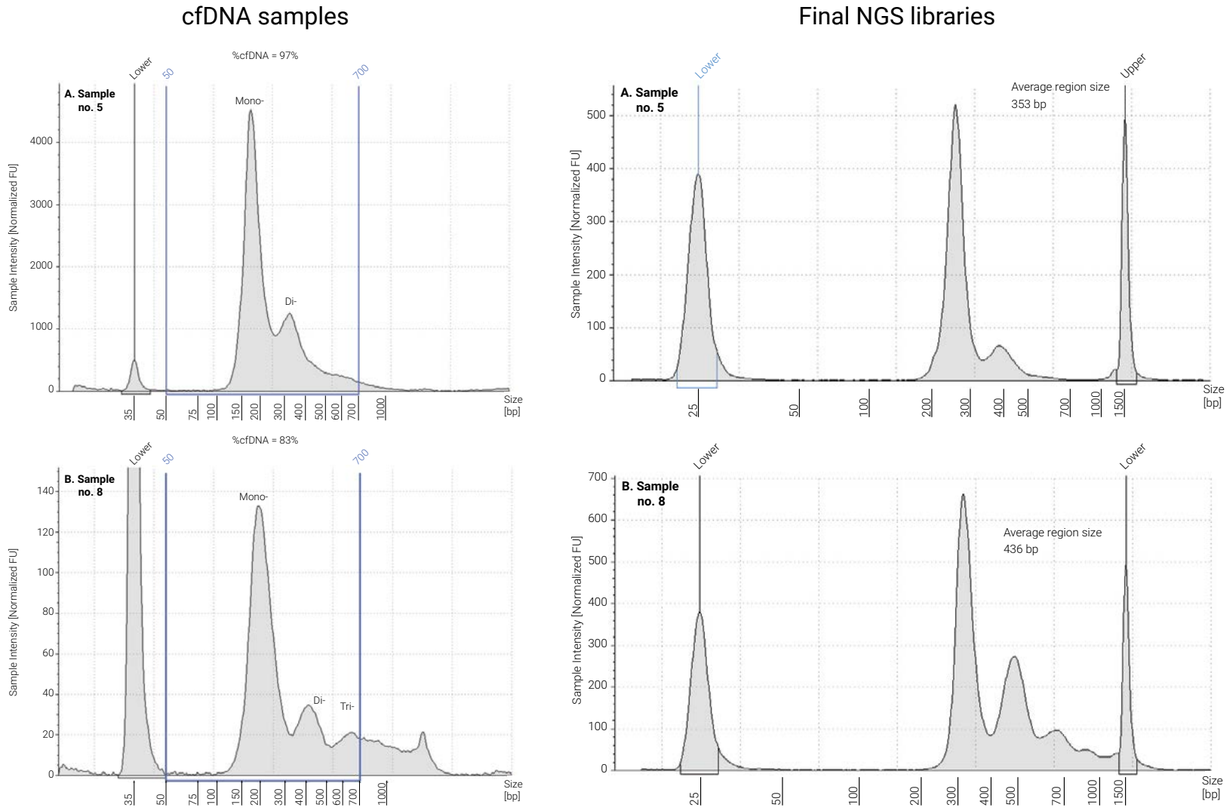
Read the full application note

Quality Control of Cell-free DNA Samples Analyzed with Next-Generation Sequencing

Application note

Abstract

Cell-free DNA (cfDNA) has become an important input material for NGS as a result of the noninvasive collection methods from blood and urine. However, analysis of cfDNA can introduce challenges due to its low concentration and possible contamination from high-molecular weight DNA, both necessitating reliable quality control. The Agilent 4200 TapeStation system is a vital quality control (QC) tool in NGS workflows. The TapeStation systems and Cell-free DNA ScreenTape assay provide a %cfDNA quality metric for determining the quality of input cfDNA for downstream processes. As part of the German Cancer Research Center (Deutsches Krebsforschungszentrum), the Sample Processing Lab collaborated with the Genomics and Proteomics Core Facility to track 13 cfDNA samples with the TapeStation system, from initial QC with the %cfDNA metric (see Figure), through the NGS workflow, to the final sequencing results. All 13 samples reported a %cfDNA greater than 83%, with successful library preparation and sequencing metrics.



Read the full application note

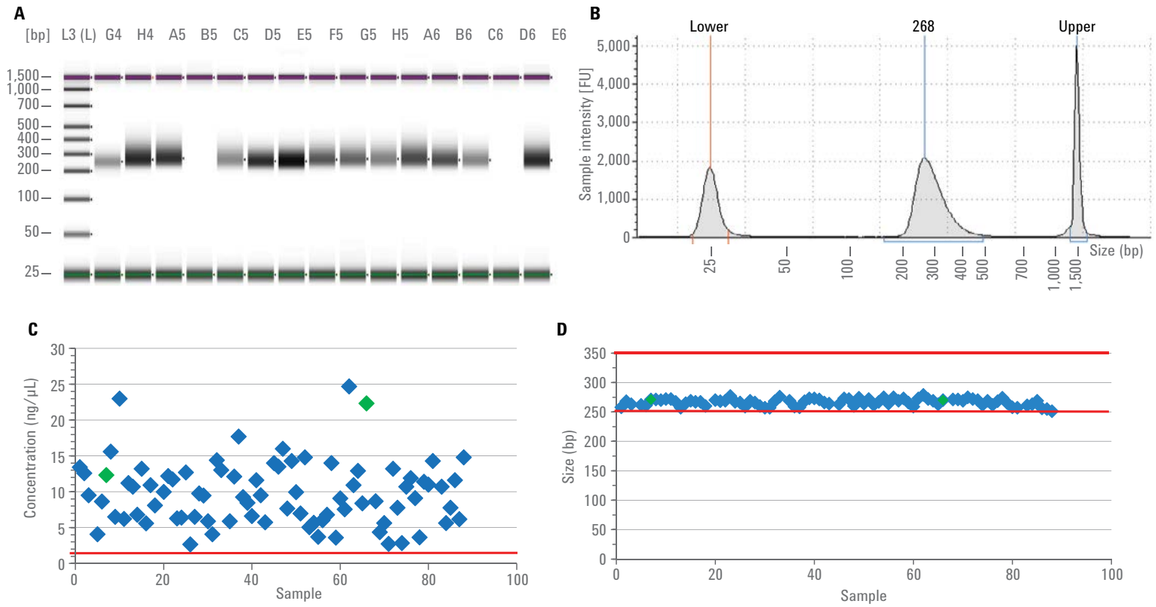
Use of the Agilent 4200 TapeStation System for Sample Quality Control in the Whole Exome Sequencing Workflow at the German Cancer Research Center (DKFZ)

Application note

Abstract

The 4200 TapeStation system was used for quality control of the final NGS libraries. These were expected to be sized between 250 and 350 bp with a minimum concentration of 2 ng/µL. In a gel view of 15 samples, lane B5 and D6 show negative controls (A). B shows an example of an electropherogram of one sample. The distribution of the concentration for all 80 samples plus eight controls is illustrated in C. The two positive controls are shown as green symbols. The red lines indicate the recommended concentration threshold (2 ng/µL). The maximum peak size for all 80 samples plus eight controls is displayed in D. The two positive controls are shown as green symbols.

The red lines indicate the recommend size range (250 to 350 bp).The analysis of the final NGS libraries with the 4200 TapeStation system confirmed successful DNA library preparation for all 80 samples and the six positive control samples.



Read the full application note

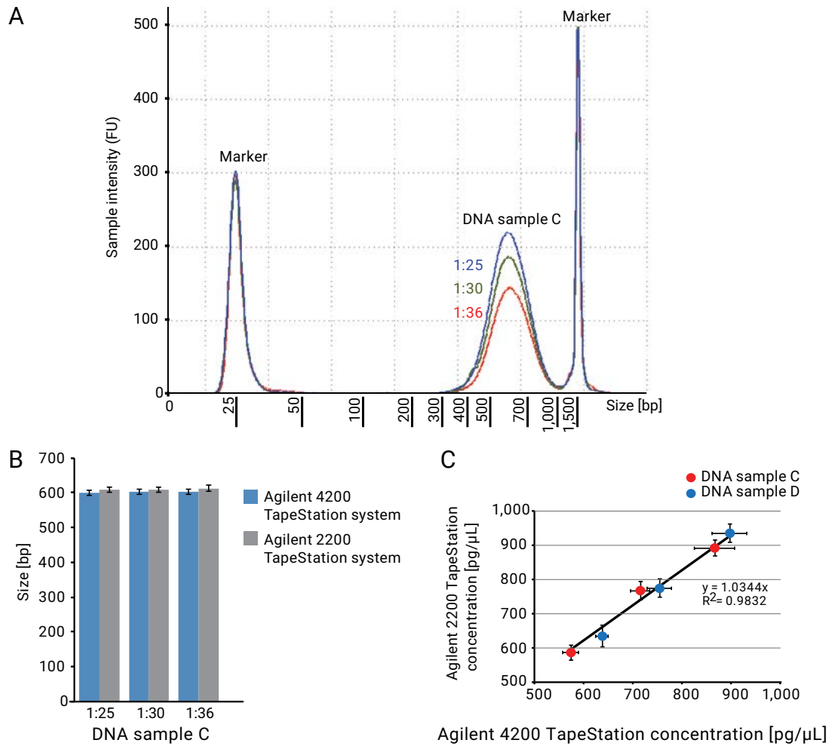
Evaluating the Agilent 4200 TapeStation System for High Throughput Sequencing Quality Control

Application note

Abstract

Quality control of NGS libraries is key to the success of any sequencing run. The D1000 ScreenTape and High Sensitivity D1000 ScreenTape assays can be used for quality control, providing DNA sizing, and quantification. The bar chart shows the sizing results of a sample in 3 dilutions analyzed with the High Sensitivity D1000 ScreenTape assay on both the 2200 and 4200 TapeStation platforms. DNA concentration determined with the 4200 TapeStation system is plotted against the concentration measured with the 2200 TapeStation system for the High Sensitivity D1000 ScreenTape assay.

The data demonstrates that results obtained with the Agilent High Sensitivity D1000 assay using both TapeStation systems are directly comparable and highly reproducible.



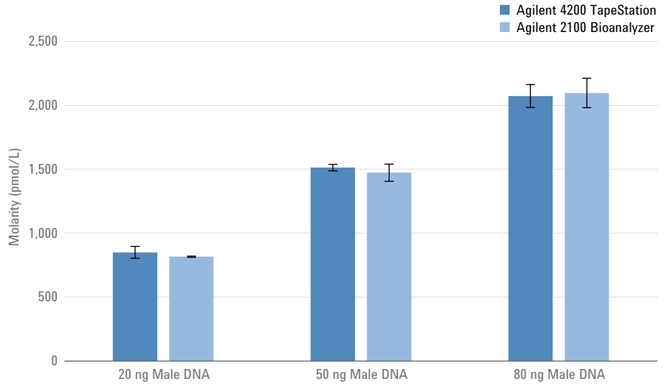
Read the full application note

Quality Control for Agilent SureSelect QXT WGS Library Preparation

Application note

Abstract

For multiplex sequencing, SureSelect^{QXT} whole-genome libraries are pooled so that each index-tagged sample is present in equimolar amounts in the final pool. The 4200 TapeStation and 2100 Bioanalyzer systems provide molarity and quantification data along with the sizing information in the region table of the software. For each library generated by various gDNA input amounts, the molarity was plotted in a graph comparing both systems. The data summarized in the table demonstrates that sizing and quantification of amplified libraries with the High Sensitivity D5000 ScreenTape assay match the results of the High Sensitivity DNA assay of the 2100 Bioanalyzer system.



Starting material		Average size (bp)		Region molarity (pmol/L)	
		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

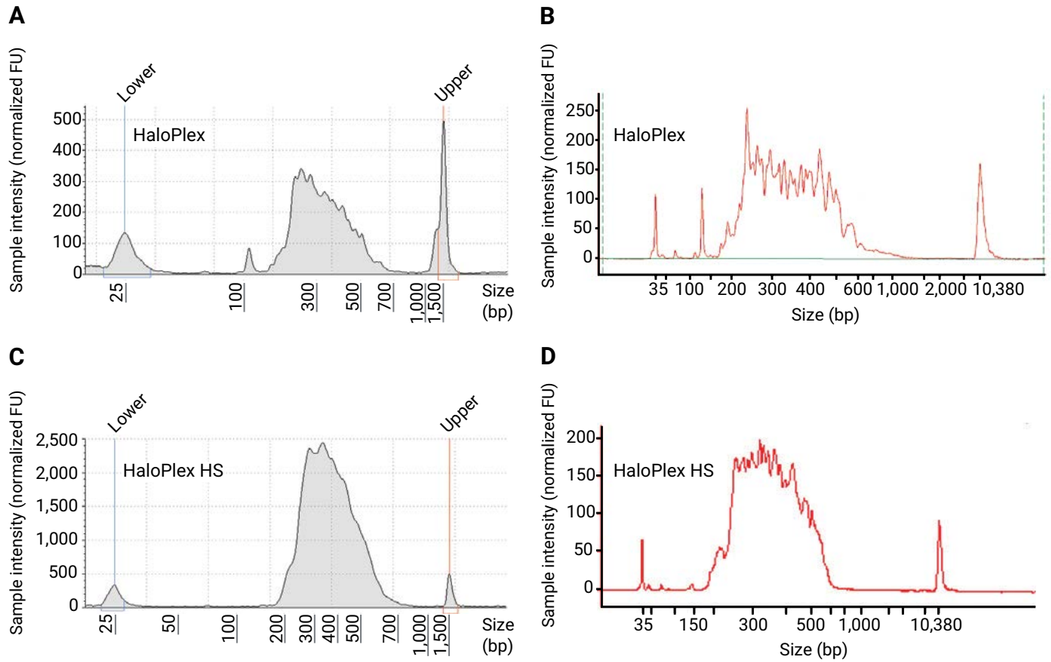
Read the full application note

Sample Quality Control in Agilent NGS Solutions

Application note

Abstract

HaloPlex and HaloPlex HS target enrichment technology uses an amplicon-based approach. The final libraries of HaloPlex and HaloPlex HS workflows show a profile with a characteristic smear in the range of 175 to 625 bp, (A) and (B), and 190 to 545 bp respectively (C) and (D). The appearance of the profile may vary due to specific library designs and the overall quality of the input material. The electropherogram should be checked for the presence of artefactual peaks with sizes less than 150 bp, as these are related to primer dimers that can cluster and consume sequencing capacity. If the primer dimer peak is greater than 10% of the total product, an additional cleanup step with AMPure beads is recommended.



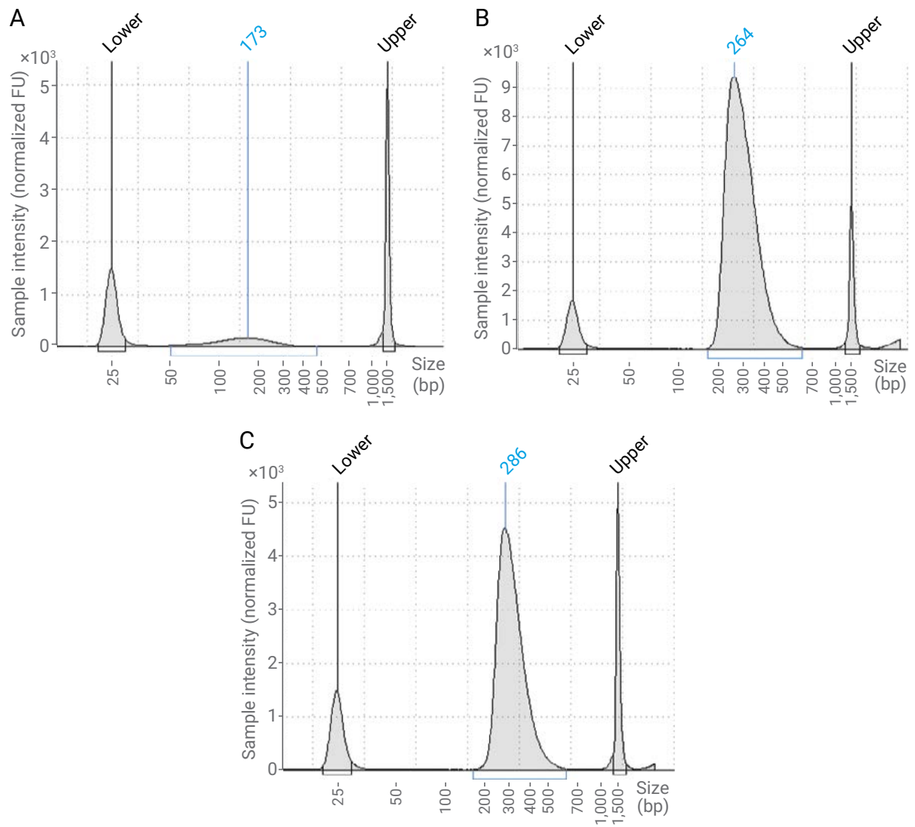
Read the full application note

Quality Control in Illumina Sequencing Workflows Using the TapeStation System

Application note

Abstract

NGS target enrichment enables a detailed analysis of specific regions to identify causal genetic variants of complex conditions. The SureSelect XT protocol is designed to create libraries with enriched targeted regions of the genome for sequencing with Illumina platforms. The two intermediate QC steps include evaluation of a smear size after shearing (A) and before capturing (B). These steps can be carried out using the D1000 ScreenTape assay. The expected size range of the maximum peak of sheared DNA is 150 to 200 bp. For precapture library, a larger maximum peak size of 225 to 275 bp is expected due to adapter ligation. The last QC step qualifies the final library before pooling (C). Another size shift is expected as a result of adding index sequences. The peak maximum of the final library is expected to be between 250 and 350 bp. A minimum concentration of 2 ng/µL is expected for successfully generated final libraries.



Read the full application note

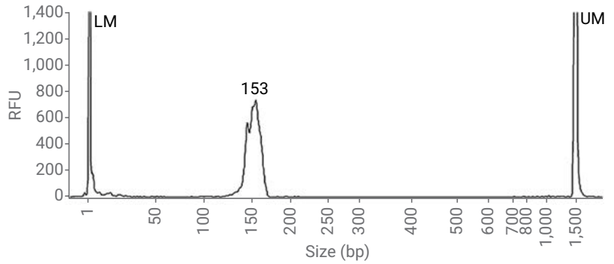
Comparison of the Agilent HS Small Fragment Kit and Agilent HS NGS Fragment Kit on the Fragment Analyzer Systems

Application note

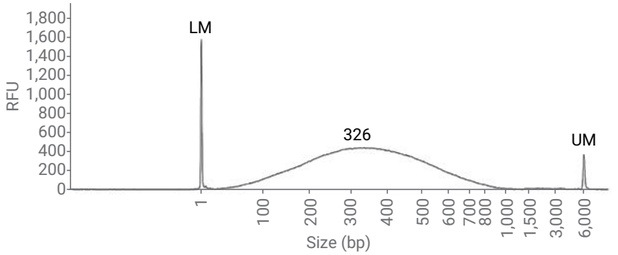
Abstract

The quality of NGS libraries is crucial to successful sequencing results. The Fragment Analyzer systems offer easy analysis of sheared genomic DNA (gDNA) and libraries with the HS NGS Fragment kit (1-6000 bp) and the HS Small Fragment kit. The HS NGS Fragment kit (C and D) analyzes larger smears and fragments up to 6,000 bp, while the HS Small Fragment kit (A and B) focuses on smaller sizes up to 1,500 bp. The FA 12-Capillary Array Ultrashort 22 cm decreases run time by 10 to 20 minutes compared to the standard FA 12-Capillary Array Short, 33 cm. The size and concentration of several DNA smears were compared between both kits and the short and ultrashort arrays. Library sizing and quantification remained consistent between the short (A and C) and ultrashort (B and D) arrays and the two kits. The HS Small Fragment kit and the HS NGS Fragment kit can be used interchangeably for sizing and quantification of NGS libraries, as long as the sample fits within the sizing range of the kit.

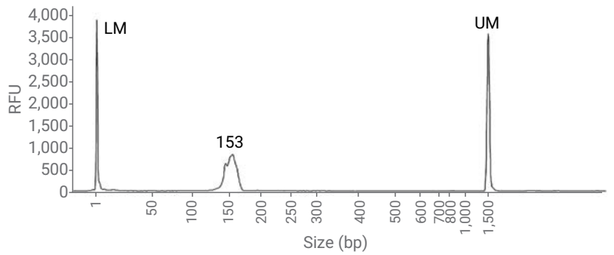
A. HS Small Fragment kit, short array, sample #1



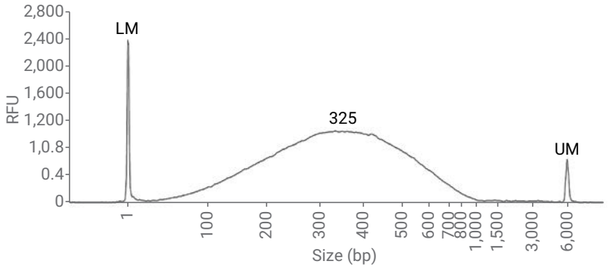
C. HS NGS Fragment kit, short array, sample #3



B. HS Small Fragment kit, ultrashort array, sample #1



D. HS NGS Fragment kit, ultrashort array, sample #3



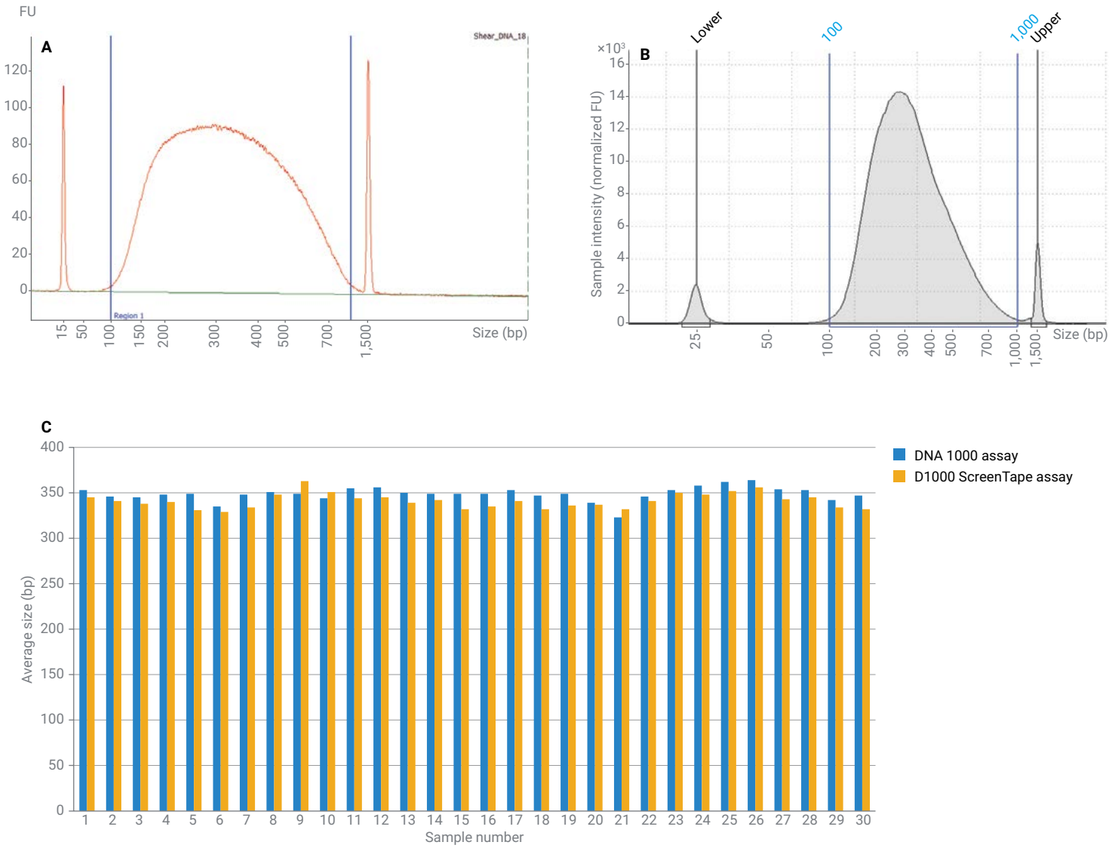
Read the full application note

Monitoring Library Preparation for Next-Generation Sequencing in Systems Biology Omics Analysis

Application note

Abstract

Frequently, the first step of a library preparation protocol is the fragmentation of gDNA by shearing with an ultrasonicator. Optimal shearing in NGS workflows can be verified by evaluating the size distribution and electropherogram pattern of fragmented DNA samples using the 2100 Bioanalyzer (A) and the 4150 TapeStation systems (B) with the DNA 1000 kit and D1000 ScreenTape assays, respectively. Electropherograms of sheared DNA in this example display an even size distribution with no undesirable shouldering. The fragmented DNA samples show a maximum peak size between 260 and 310 bp on both systems, verifying optimal shearing (C). The size of the sample at this QC step can be compared to the size of sample after adapter ligation in the library preparation workflow, at which point a shift in size is expected. Overall sizing results of the 2100 Bioanalyzer and the 4150 TapeStation systems correlated highly with an average deviation of 2.2% for all 30 samples analyzed.



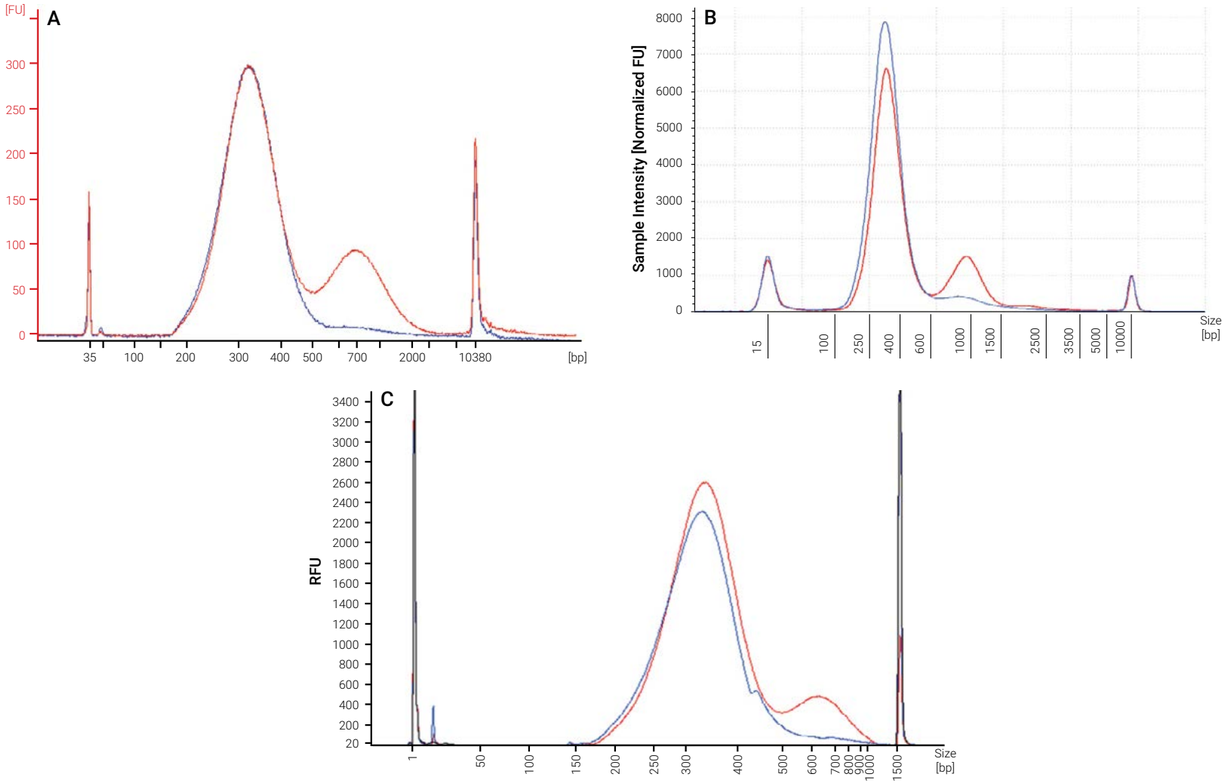
Read the full application note

Quality Control of NGS Libraries with Daisy Chains

Application note

Abstract

The Bioanalyzer, TapeStation, and Fragment Analyzer systems with dedicated high sensitivity assays allow unambiguous detection of daisy chains and accurate sizing of the target library peak. Two KAPA HyperPlus libraries with different levels of amplification were analyzed on the Bioanalyzer (A), TapeStation (B), and Fragment Analyzer (C) systems using the High Sensitivity DNA kit, High Sensitivity D5000 ScreenTape assay, and HS Small Fragment kit respectively. As shown in the overlays, both libraries (blue – library 1, red – library 2) contained the desired library peak with a pronounced secondary peak in library 2. The excessive amplification of library 2 resulted in the formation of daisy chains, which were observed as an additional higher molecular weight peak in all electropherograms. The daisy chains migrated slower through the gel matrix and were easily detected by all Agilent automated electrophoresis instruments using the respective assays. In this application note, we provide a recommendation for which assay to employ to reliably visualize daisy chain products in next-generation sequencing libraries. Furthermore, we emphasize the consistency between the instruments and reproducibility of analysis confirmed by results on a series of double dilutions.



Read the full application note

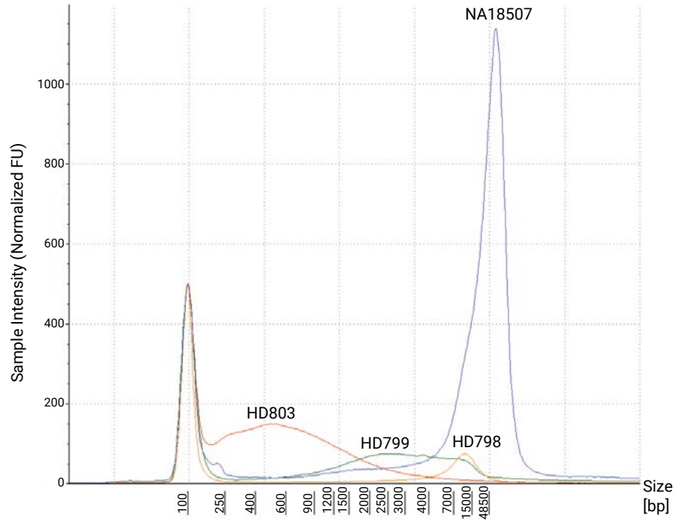
Quality Control of Magnis SureSelect XT HS Workflows with Agilent Automated Electrophoresis Solutions

Application note

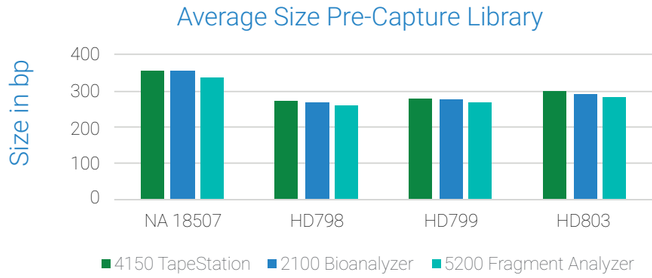
Abstract

The Magnis NGS Prep system is an automated NGS library preparation solution for the SureSelect XT HS system. It addresses challenges of manual library preparation, such as hands-on time, expertise, optimization, and validation for diverse applications. Performing QC steps and quantification on the starting material, the materials derived from intermediate steps (optional), and the final library is beneficial in ensuring reliability and overall success of the sequencing data. QC steps can be performed with the automated electrophoresis portfolio of instruments, including the Bioanalyzer, TapeStation, and Fragment Analyzer systems. gDNA was assessed with the Genomic DNA ScreenTape assay on the 4150 TapeStation system for overall integrity and size in high-quality gDNA, and mildly, moderately, and highly degraded FFPE samples (A). This assay applies a quality score, the DNA integrity number (DIN), to each sample. The score is used to optimize the fragmentation step and determine the amount of input DNA to be used in library preparation. Post-capture libraries were assessed on all three platforms and displayed similar sizing (B).

A



B



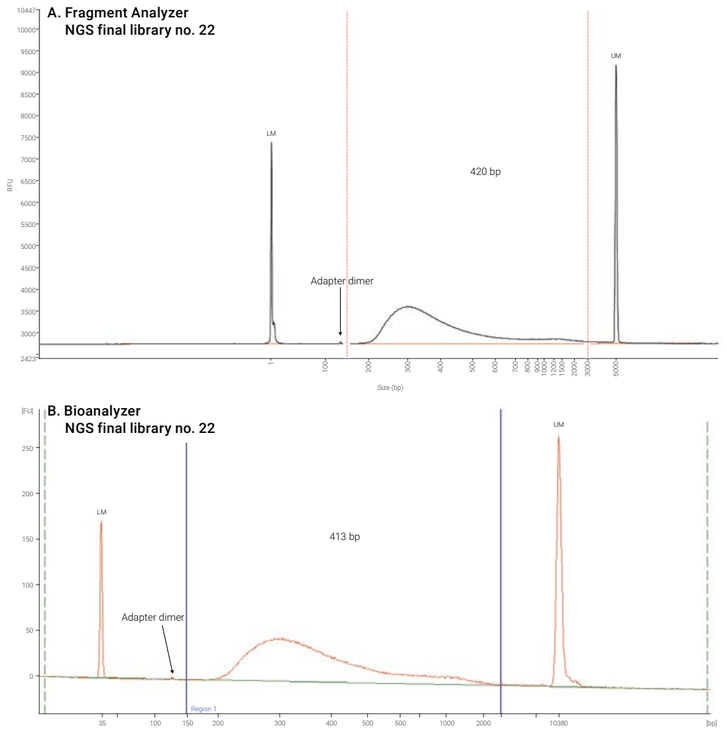
Read the full application note

Comparison of DNA Sample QC for NGS Workflows with the Agilent Fragment Analyzer and Bioanalyzer Systems

Technical overview

Abstract

In this technical overview, precapture and final libraries were compared on both the Agilent Fragment Analyzer and Bioanalyzer systems with their corresponding DNA assays to demonstrate data equivalency across platforms. The DNA assays selected have similar analytical specifications, allowing for seamless comparison between the instruments. Both systems support standard-sensitivity (SS) and high-sensitivity (HS) kits, covering a wide range of sample concentrations that occur at different QC checkpoints throughout library preparation. Starting DNA material can vary greatly in concentration, depending on whether its source is from fresh material or ancient samples. The HS kits are ideal for conservation of low-concentration samples, and can be used at all recommended QC checkpoints. Alternatively, the SS kit has the advantage of analyzing high-concentration samples while eliminating time-consuming dilution steps. Having the option of both SS and HS kits simplifies the library preparation workflow. The SS and HS kits are compared in this technical overview.



Read the full technical overview

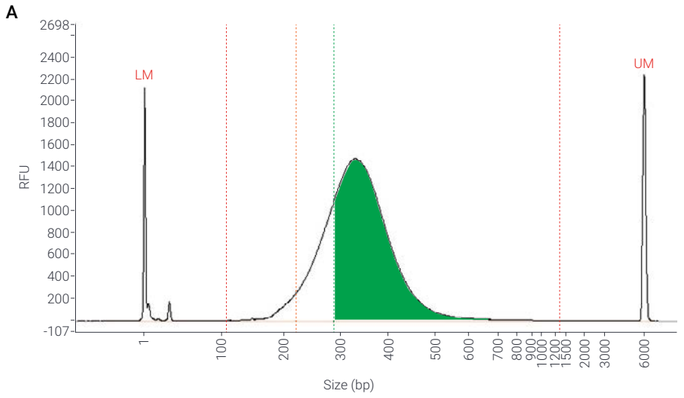
Preventive Control of Sequencing Through the Insert with the Agilent 5200 Fragment Analyzer System

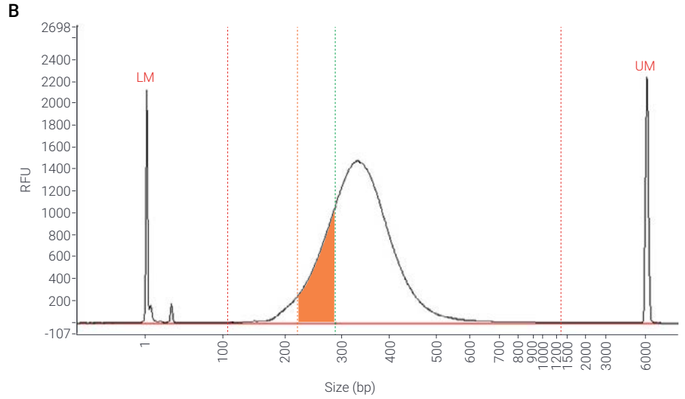
Application note

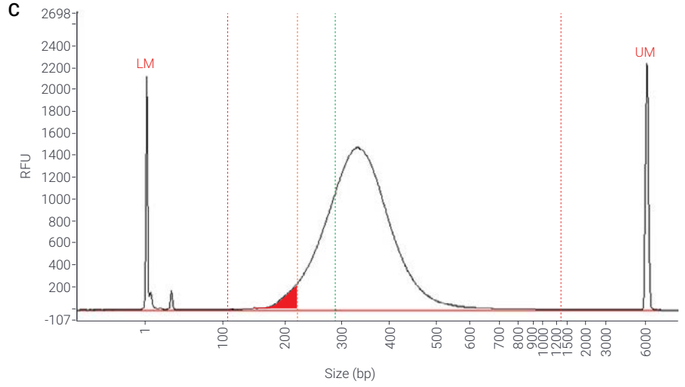
Abstract

The Agilent Fragment Analyzer systems, together with the Agilent NGS Fragment kits and the companion Agilent ProSize data analysis software, offer a well-established solution for sample quality control (QC) in next-generation sequencing (NGS) workflows. This application note expands the capabilities of the ProSize smear analysis, and demonstrates how its functionality can be further applied to avert a specific sequencing issue called sequencing through the insert. This data is then introduced into the sequencing read and, in some cases, can increase the background noise, decreasing the quality of the overall sequencing run. The percent total value of the ProSize software allows a user to estimate the percentage of the library that will be sequenced through the insert based on the planned sequencing run method and electrophoretic profile of the library. Easy and comprehensive smear analysis enables the user to determine an optimal read length, and thereby minimize the number of unwanted bases present in the sequencing reads, saving expensive reagents and time for auxiliary data processing.

ID	Range	ng/µL	% Total	nmole/L	Avg. Size	%CV
A2: S1 - 2 ng/µL	110 bp to 1350 bp	2.3296	98.7	11.5454	332	23.30
	220 bp to 1350 bp	2.2339	94.6	10.8748	338	21.67
	286 bp to 1350 bp	1.7412	73.7	7.9547	360	18.82







Read the full application note

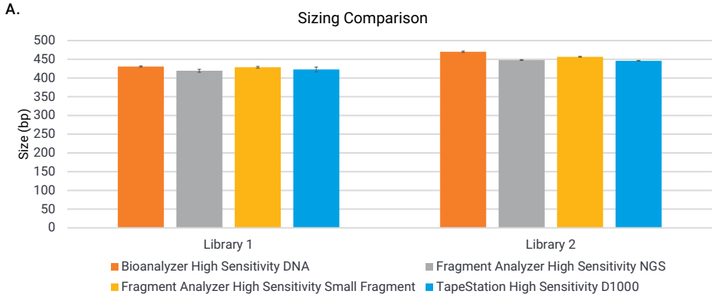
Quality Assessment of NGS Libraries using Agilent Automated Electrophoresis Systems

Technical overview

Abstract

The Agilent automated electrophoresis instruments, including the Bioanalyzer, Fragment Analyzer, and TapeStation systems, are well-suited for QC of NGS libraries, providing high-quality quantification, qualification, and sizing of nucleic acids to allow for confident assessment of samples throughout the NGS workflow. Each instrument offers specific benefits suited to meet a variety of individual laboratory needs, such as throughput, sensitivity, speed, and resolution. To demonstrate the equivalency of the systems to each other, NGS libraries were compared across each instrument. Each instrument offers a versatile assay portfolio that covers broad sizing and concentration ranges. The DNA analysis kits chosen for analysis of the NGS libraries in this technical overview have similar specifications, with a sizing range well-suited for the NGS smear, allowing for comparison between the instruments. This technical overview highlights the capability of each system to accurately size and quantify NGS libraries.

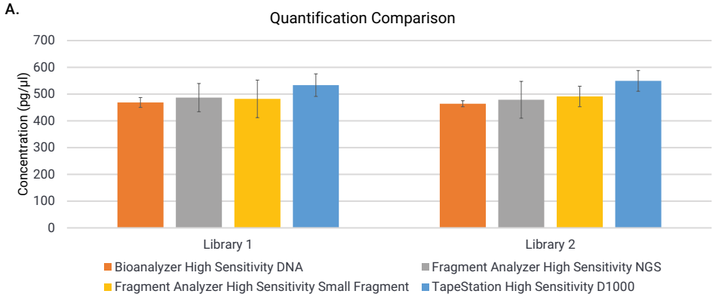
A.



B.

	Average Size (bp)		%CV	
Instrument and Kit	Library 1	Library 2	Library 1	Library 2
Bioanalyzer High Sensitivity DNA	431	470	0.35	0.43
Fragment Analyzer High Sensitivity NGS	419	448	0.96	0.22
Fragment Analyzer High Sensitivity Small Fragment	429	457	0.54	0.25
TapeStation High Sensitivity D1000	423	446	1.54	0.22

A.



B.

	Average Conc. (pg/µL)		%CV		%error	
Instrument and Kit	Library 1	Library 2	Library 1	Library 2	Library 1	Library 2
Bioanalyzer High Sensitivity DNA	469	464	3.95	2.45	17.20	17.74
Fragment Analyzer High Sensitivity NGS	487	479	10.86	14.41	13.99	15.13
Fragment Analyzer High Sensitivity Small Fragment	482	491	14.56	7.81	14.84	12.91
TapeStation High Sensitivity D1000	533	549	7.91	7.05	5.77	2.60

Read the full technical overview

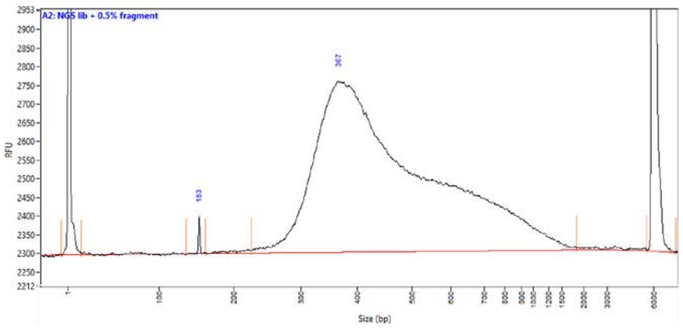
Detection of Adapter Dimers in NGS Libraries with the Agilent Fragment Analyzer and TapeStation Systems

Technical overview

Abstract

During the process of library preparation, DNA fragments are ligated to a known sequence, or adapter. These adapters allow the DNA fragments to bind to the flow cell during the sequencing run. Even small amounts of adapter dimers in a library can contribute to detrimental sequencing results. The presence of adapter dimers can affect the accuracy of library quantification, leading to suboptimal flow cell loading and reduced clustering efficiency. Smaller fragments, such as dimers, are also preferentially sequenced over larger fragments, so even low levels of adapter dimers could contribute to issues such as reduced output, lower diversity, and decreased genome coverage. While the optimal amount of adapter dimer in a library is zero, there are thresholds of adapter dimer in a library that may be present and still generate satisfactory sequencing results. Thus, it is highly recommended to minimize and remove adapter dimers from the library before sequencing. This technical overview highlights the sensitivity and resolution of the Agilent Fragment Analyzer and TapeStation systems to detect adapter dimers even lower than the recommended threshold of 0.5% and deliver an accurate assessment of the percentage of adapter dimer in the library.

0.5% Fragment



0.5% Fragment

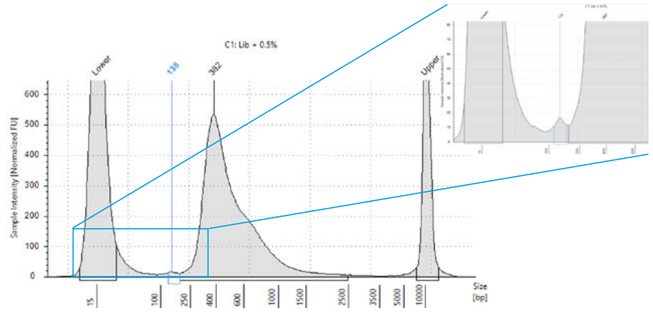


Table 2. Reported size and percentage of adapter dimer present within an NGS library analyzed on the Agilent 5200 Fragment Analyzer system and the Agilent 4200 TapeStation system.

	Fragment Analyzer HS NGS Kit		TapeStation HS D5000 ScreenTape Assay	
Expected Adapter Dimer	**Fragment Size (bp)**	**% (Conc.)**	**Fragment Size (bp)**	**% Integrated Area**
5%	153	4.2%	124	4.7%
1%	154	0.9%	138	1.3%
0.5%	153	0.5%	138	0.8%
0.1%	154	0.1%	Not detected	Not detected

Read the full technical overview

Want to Explore Even More Resources?

Performing quality control of your nucleic acid samples with Agilent automated electrophoresis solutions can help advance your next-generation sequencing workflows while conserving valuable time and resources.

Browse all of our automated electrophoresis solutions at:
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