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Agilent 2100 Bioanalyzer System
Agilent 4200 TapeStation System

Applications for DNA, RNA, and Protein Analysis

Application Compendium

Agilent 2100 Bioanalyzer System

Agilent 4200 TapeStation System

Applications for DNA, RNA, and Protein Analysis

Application Compendium

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- Genomics
- Proteomics
- Metabolomics
- Bioinformatics
- Pharmaceutical research

Contents

Agilent 2100 Bioanalyzer system

Introduction Agilent 2100 Bioanalyzer system 10
Microfluidic electrophoresis 11

DNA analysis 14

Next-generation sequencing
DNA library QC in target enrichment and next-gen sequencing workflows 14
Sizing and quantitation of DNA libraries and fragmented DNA 15
Quality control of FFPE DNA samples 16
Analysis of limited DNA material on the Pippin Prep system 17

PCR product analysis
Optimize QPCR assay design 18

Food analysis
Estimation of non-basmati rice amounts in basmati rice products 19
Strawberry and raspberry fruit differentiation 20
Detection of non-fish species with PCR-RFLP 21

RNA analysis

Analysis of total RNA
Standardization of RNA quality control 22
Quality control with DV_{200} evaluation of FFPE RNA samples 23
Assessing integrity of plant RNA 24
Assessing integrity of insect RNA 25

Analysis of Small RNA
Analysis of miRNA content in total RNA samples 26
Analysis of small RNAs from *Drosophila* Schneider 27

Genome editing 28
Guide RNA analysis for CRISPR-Cas9 genome editing 28
Protocol optimization for CRISPR-Cas9 workflows 29

Protein analysis 30
Protein purification
Optimization of on-column cleavage 30
Analysis of modified proteins
Analysis of PEGylated proteins 31
High sensitivity protein analysis
Detection of low abundant proteins 32
Highly specific and sensitive alternative to western blotting 33
Quantitation of low protein abundance with an internal standard 34
Antibody analysis
Analysis of IgG2 under nonreducing conditions 35
IgG2 analysis under reducing conditions 36
Food analysis
Rapid wheat varietal identification 37
Protein analysis in milk 38

Contents

Agilent 4200 TapeStation system

Introduction Agilent 4200 TapeStation system 39

DNA analysis 42

Next-generation sequencing

Impact of gDNA Integrity on the outcome of DNA methylation studies 42

Analysis of DNA from FFPE tissue 43

Quality control of DNA from FFPE tumor samples 44

Quantitation of amplified SureSelect^{QXT} whole genome libraries 45

Quality control of final NGS libraries 46

Analysis of NGS libraries – equivalence of the 4200 TapeStation and 2200 TapeStation system 47

PCR product analysis

Quality control of single cell DNA samples 48

Genomic DNA

Optimization of gDNA Extraction from FFPE tissue 49

Equivalence of the 4200 TapeStation and 2200 TapeStation systems 50

Quantitation of isolated genomic DNA (gDNA) samples 51

Quantity and integrity analysis of genomic DNA for next generation sequencing 52

Integrity analysis of genomic DNA from bacterial sources 53

RNA analysis 54

Gene Expression Analysis

Impact of RNA degradation on qPCR experiments 54

Analysis of total RNA

Equivalence of the 4200 TapeStation and 2200 TapeStation system RNA integrity number 55

Equivalence of the 4200 TapeStation and 2200 TapeStation system quantitation 56

Comparison of the RNA integrity analysis on 2100 Bioanalyzer system with 2200 TapeStation system 57

Equivalence of RNA analysis on 2200 TapeStation and 2100 Bioanalyzer system 58

RNA Quantitation

RNA quantitation with the TapeStation system in comparison with spectrophotometric and fluorescence based measurements 59

RNA Purity

Detection of genomic DNA contamination 60

Analysis of FFPE RNA

Simplified DV_{200} Evaluation with the 4200 TapeStation 61

LIMS Integration 62

LIMS integration of the Agilent 4200 TapeStation system 62

Literature 63

Overview Application Notes and other publications

Agilent 2100 Bioanalyzer system

Learn more at:
www.agilent.com/genomics/bioanalyzer

A wide range of applications

The Agilent 2100 Bioanalyzer system is an easy-to-use benchtop platform with ready-to-run kits for a wide range of applications.

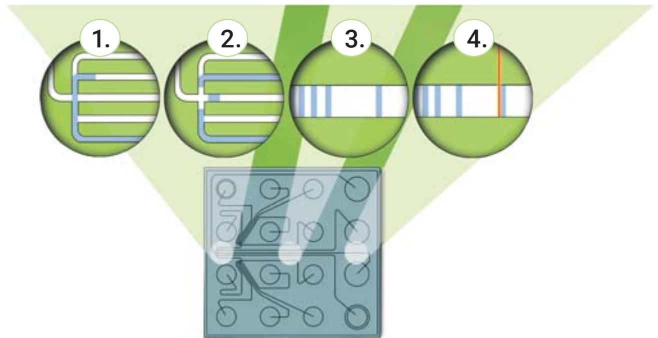
- **DNA size and quantity**
  High resolution separation and quantitation of DNA down to pg/µL sensitivity.
- **RNA quality check with RIN**
  An established standard for RNA analysis offering analysis of total RNA, mRNA, and small RNA. Objective integrity analysis of total RNA is provided with RIN (RNA Integrity Number) and with the DV_{200} metric (for FFPE RNA samples prior to RNA sequencing studies).
- **SDS-PAGE replacement for protein analysis**
  The fast and reliable way to determine the quantity and purity of proteins from Coomassie down to silver stain sensitivity.

When combined with any Agilent kit, you will discover how microfluidic technology can revolutionize your laboratory. Microfluidics utilizes interconnected networks of microchannels and wells for the analysis of various sample types. The technology reduces overall space and volume requirements, and allows online integration of many workflow steps, such as sample enrichment, separation, staining, destaining, and detection.

Advantages of microfluidics include dramatically reduced sample and reagent consumption, significantly faster analysis times, and reduced hands-on work during sample preparation and data analysis. The Agilent 2100 Bioanalyzer system represents the first microfluidic platform for the electrophoretic analysis of DNA, RNA, and proteins. This versatility makes the 2100 Bioanalyzer system an indispensable tool for the molecular biologist and biochemist.

Microfluidic electrophoresis increases the quality and efficiency of your analysis

Microfluidic electrophoresis has many advantages over conventional methods, such as gel electrophoresis.



Principle of operation
1. The sample moves through the microchannels from the sample well.
2. The sample is injected into the separation channel.
3. Sample components are electrophoretically separated.
4. Components are detected by their fluorescence and translated into gel-like images (bands) and electropherograms (peaks).

One of the many benefits the Agilent 2100 Bioanalyzer system has over conventional bioanalytical methods is the elimination of time consuming procedures—so you can enjoy standardized handling and interpretation of data. It simplifies the process of data gathering and analysis down to three quick and easy steps:

Load sample, run analysis, view data

– The Agilent 2100 Bioanalyzer system and the various **DNA kits** are the tools of choice for automated sizing and quantitation of products generated by RT-PCR and any type of multiplex PCR with unprecedented accuracy and reproducibility. It not only provides the detection of the presence or absence of a PCR product, but also offers quantitation of this product and detection of unspecific amplification. Therefore, the Agilent 2100

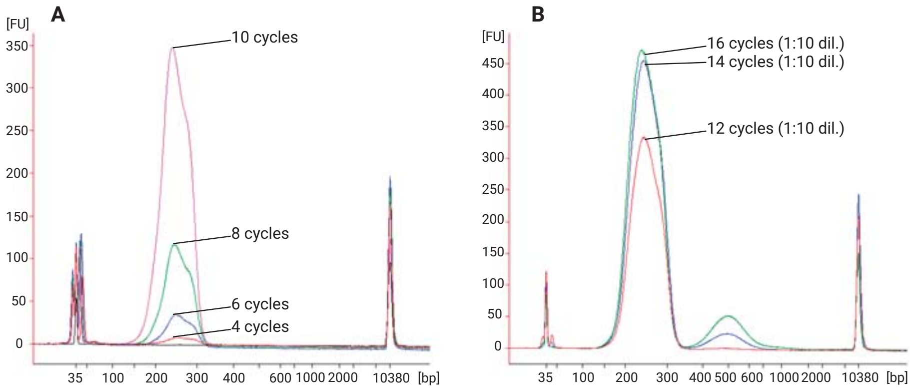
Bioanalyzer system helps to optimize PCR reactions for gene expression, sequencing, cloning, and accurate analysis of restriction digests.

- The **High Sensitivity DNA kit** provides sizing and quantitation of DNA fragments and DNA smears in the 50 to 7000 bp size range down to pg/µL sensitivity. This is especially useful 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.
- Due to the omnipresence of RNases and the instability of RNA, integrity checks and sample quantitation are essential steps before any RNA-dependent experiment. The 2100 Expert software generates the unambiguous RNA Integrity Number (RIN), provides a quantitation estimate, calculates ribosomal ratios of total RNA samples, and automatically detects ribosomal RNA contamination in mRNA. The **RNA 6000 Nano kit** is a well-established standard for RNA sample quality control. The **RNA 6000 Pico kit** allows detection of RNA degradation with sample amounts down to 200 pg of total RNA.
- The high resolution **Small RNA kit** allows separation, verification, and optimization of miRNA after extraction procedures. It is also often used for sample quality control of guide RNAs for CRISPR experiments. By consuming only 1 µL sample, even pg amounts of purified small RNA can be measured reproducibly and comparably within 30 minutes. By effectively staining single- and double-stranded oligonucleotides at the same time, the assay is a versatile tool.

- The **Protein 80 and Protein 230 kits** provide fast and easy analysis of a wide range of samples, whether expressed recombinant proteins, purified proteins, stability studies, or an antibody quality check. Microfluidic electrophoresis provides size, purity, and concentration information for 10 protein samples in less than 30 minutes. This approach eliminates handling of SDS-PAGE gels, staining, or imaging steps.
- With the **High Sensitivity Protein 250 kit**, it is possible to analyze proteins down to 1 pg/µL (on chip), which is equivalent or superior to silver stain SDS-PAGE. It provides quantitation over a dynamic range of up to four orders of magnitude with the reproducibility and ease-of-use only associated with the Agilent 2100 Bioanalyzer system.
- The optional **Agilent 2100 Security Pack software** ensures full 21 CFR Part 11 compliance of your 2100 Bioanalyzer system for regulated environments such as pharmaceutical QA/QC labs or manufacturing. It addresses requirements, such as electronic signatures, audit trails, and user authentication. Along with IQ and OQ support services and declarations of conformity for all components offered for all assays and kits, your Agilent 2100 Bioanalyzer system will be compliant in no time.

Next-generation sequencing

DNA library QC in target enrichment and next-gen sequencing workflows



From [bp]	To [bp]	Corrected area	% of total	Average size [bp]	Size distribution in CV [%]	Concentration [pg/µL]	Molarity [pmol/L]
100	2,000	395.8	51	254	12.4	283.33	1,713.8

Quantitation after 4 PCR cycles

Kit: **High Sensitivity DNA kit**

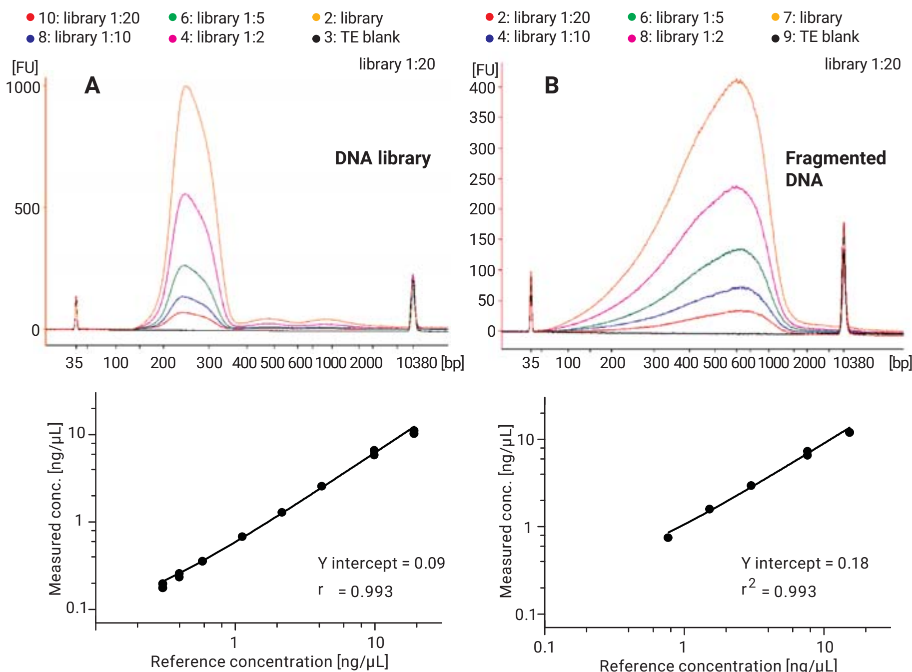
Software Assay: **High Sensitivity DNA assay**

Abstract: The High Sensitivity DNA kit was used for quality control of amplified and purified DNA samples from the post-hybridization PCR amplification step before sequencing during the SureSelect Target Enrichment workflow. The electropherograms of typical PCR amplified DNA libraries show a smear from 150 to 350 nucleotides. The key observation clearly shown in figure B, is that the quality of the PCR product is dependent on the number of PCR cycles performed. After 14 PCR cycles, an additional DNA smear at approximately 500 bp was detected in the electropherogram. The highly sensitive nature of the High Sensitivity DNA kit allowed the amplified DNA to be detected and reliably quantified, even after only four PCR cycles. Thus, the number of library PCR cycles can be reduced, removing amplification bias and significantly improving the data quality with increased accuracy.

Application note: 5990-5008EN

Next-generation sequencing

Sizing and quantitation of DNA libraries and fragmented DNA



Kit: **High Sensitivity DNA kit**

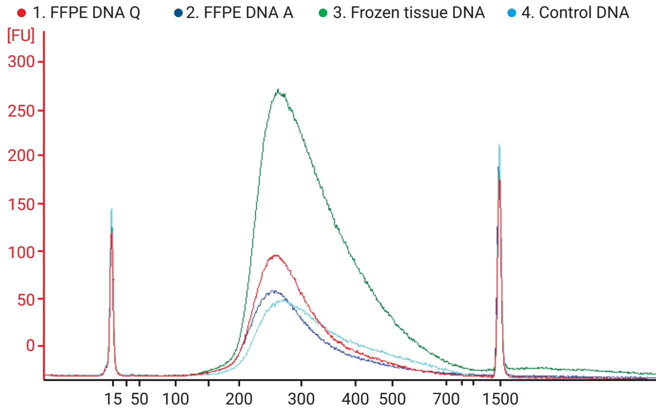
Software Assay: **High Sensitivity DNA assay**

Abstract: The High Sensitivity DNA kit provides sizing and quantitation of DNA fragments and DNA smears in the 50 to 7000 bp size range down to pg/µL sensitivity. This is especially useful 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. The analysis of a dilution series from two typical NGS samples, (A) Illumina DNA library and (B) fragmented DNA was performed. For both DNA sample types, the double logarithmic plot demonstrates an excellent linearity with r^2 = 0.993. The linear dynamic range for smear samples of the High Sensitivity DNA kit was found to be between 50–100 pg/µL and 5,000–10,000 pg/µL. This linear dynamic range depends on the library type and fragment distribution. The broad linear dynamic range of the High Sensitivity DNA kit enables the detection of less abundant products, such as PCR artifacts and impurities.

Application note: 5990-4417EN

Next-generation sequencing

Quality control of FFPE DNA samples



Sample	Average size [bp]	Peak height [bp]	Concentration [ng/µL]
FFPE DNA Q	307	264	34.8
FFPE DNA A	309	254	22.6
Frozen tissue DNA	331	264	89.5
Control DNA	348	271	23.2

Average size, peak height, and quantification of precaptured amplified libraries.

Kit: **DNA 1000 kit, High Sensitivity DNA kit**

Software Assay: **DNA 1000 assay, High Sensitivity DNA assay**

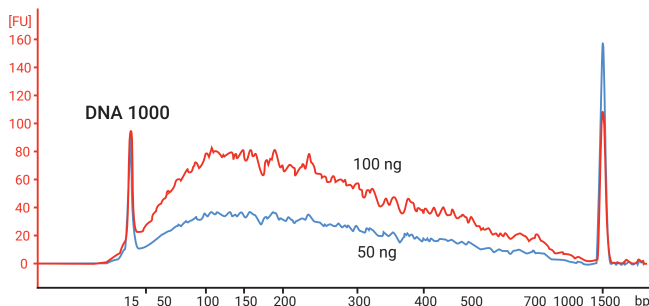
Abstract: The Agilent 2100 Bioanalyzer system was used for quality control of DNA samples from formalin-fixed paraffin-embedded (FFPE) and fresh-frozen tissues before and during the SureSelect Target Enrichment workflow. The figure shows the electropherogram overlay of precapture amplified samples after five PCR cycles run on the Agilent 2100 Bioanalyzer system with the Agilent DNA 1000 kit. Similar profiles were observed for all DNA samples. No amplification artifacts or primer dimers were seen. FFPE DNA samples gave comparable results to DNA from fresh-frozen tissue and control cell line DNA, appropriate for downstream sequencing on the Illumina platform.

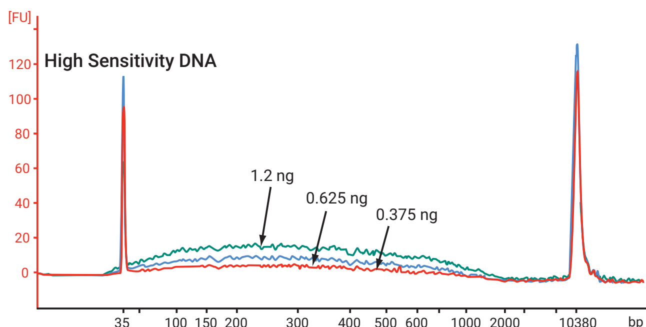
Reliable DNA electrophoresis with the Agilent 2100 Bioanalyzer system provided smear profiles and details for library statistics, such as peak heights, average smear size, size distribution, and DNA concentration.

Application note: 5991-0483EN

Next-generation sequencing

Analysis of limited DNA material on the Pippin Prep system





Kit: **DNA 1000 kit, High Sensitivity DNA kit**

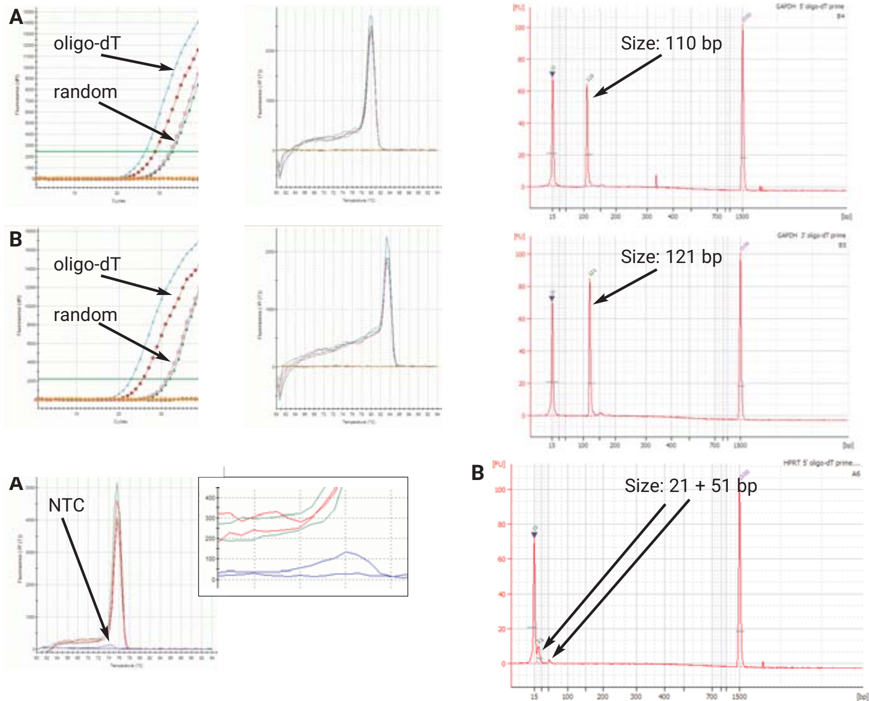
Software Assay: **DNA 1000 assay, High Sensitivity DNA assay**

Abstract: The Agilent 2100 Bioanalyzer system and the High Sensitivity DNA kit complement the Pippin Prep automated size selection workflow (Sage Science, Inc.). The figure demonstrates the DNA analysis of a restriction digest of *E. coli* genomic DNA to simulate a sheared Pippin Prep input sample. Using the High Sensitivity DNA kit allows the use of only 1.2 ng DNA to achieve a roughly equivalent signal to 50 ng on the DNA 1000 kit. Even lower DNA amounts (0.375 ng) yield reasonable electropherograms showing input size distribution. This allows tailoring fractionation settings for the Pippin Prep system, and maximizing chances for successful library construction. Afterwards, the 2100 Bioanalyzer system can be used to confirm size ranges, quality, and purity of the Pippin Prep process. The 2100 Bioanalyzer and Pippin Prep systems work well in concert to enable fine control and increase the efficiency of library generation before next-generation sequencing.

Application note: 5990-8382EN

PCR product analysis

Optimize QPCR assay design



Kit: **DNA 1000 kit**

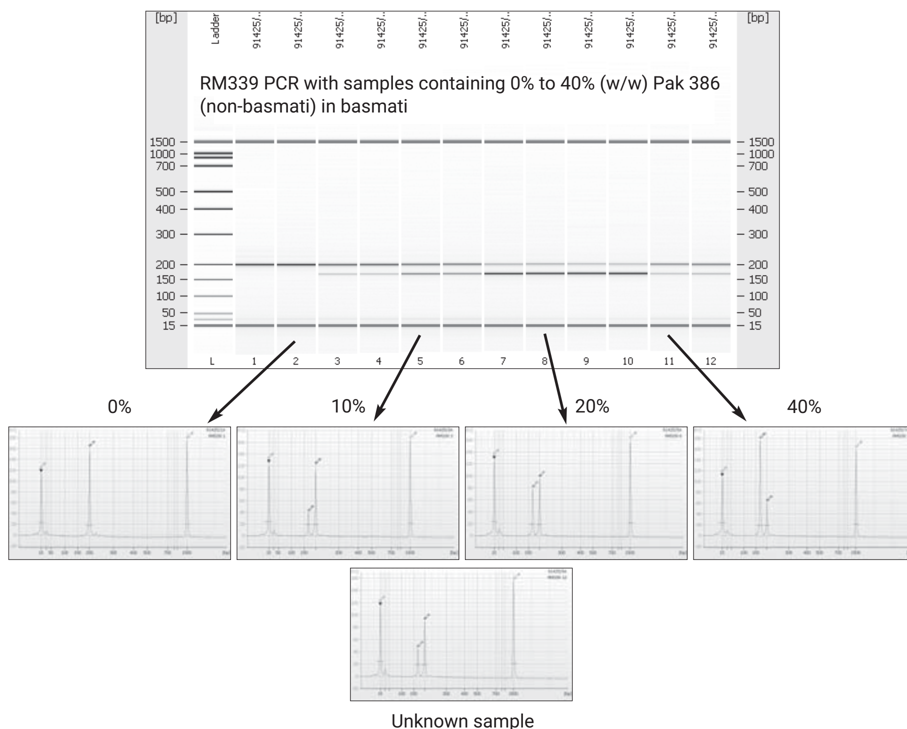
Software Assay: **DNA 1000 assay**

Abstract: To determine the best possible reverse transcription (RT) priming strategy, QPCR, and 2100 Bioanalyzer system tests were conducted for three genes (GAPDH, HPRT1, and YWHAZ) by using high-quality RNA (RIN = 10) as template. The RNA was reverse transcribed with either oligo-dT or random priming (A: 5'-assay, B: 3'-assay for GAPDH). To assess size and purity of QPCR amplicons, 1 µL of the QPCR reactions was analyzed with the DNA 1000 assay. In addition, no-template-controls (NTC) were used to assess contamination and potential primer dimer formation, since a small amount of contaminating template can lead to amplification. For the HPRT1 5' oligo-dT assay one of the NTC was positive, showing a peak in the melt curve at a similar melting temperature compared to the positive control (A). To verify that no contamination of the well has occurred, the NTC was analyzed using the DNA 1000 assay on the 2100 Bioanalyzer instrument (B). Two minor peaks could be detected at 21 and 51 bp, which are most probably related to primer and primer dimers. This highlights the high information content obtained by the 2100 Bioanalyzer system and the poor discrimination capabilities of a SYBR Green-based melt curve.

Application note: 5989-7730EN

Food analysis

Estimation of non-basmati rice amounts in basmati rice products



Unknown sample

Kit: **DNA 1000 kit**

Software Assay: **DNA 1000 assay**

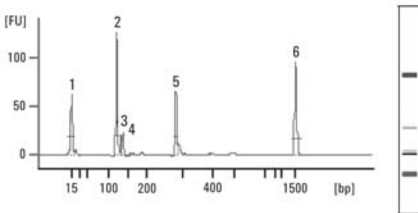
Abstract: Ensuring integrity of raw food materials, ingredients, and products is both a product quality and regulatory compliance concern. Food ingredient suppliers, manufacturers, and retailers can suffer economic and legal damages if proven to be supplying materials or products that are incorrectly labeled due to substitution or contamination. For example, EU Commission 1549/04 grants lower import tax on nine basmati rice varieties. Furthermore, the level of non-basmati rice in a basmati rice product must not exceed 7 %. Therefore it is required to identify basmati rice varieties and determine the level of non-basmati rice in basmati rice products. The 2100 Bioanalyzer system and the DNA 1000 kit can be used as a quick and cost effective analytical method to differentiate approved and nonapproved rice varieties using three primer sets and to estimate the level of non-basmati rice using reference rice admixtures. Four reference samples with 0 to 40 % non-basmati rice content were used to estimate the non-basmati amount in an unknown sample to be 10-20 %.

Application note: 5989-6836EN

Food analysis

Strawberry and raspberry fruit differentiation

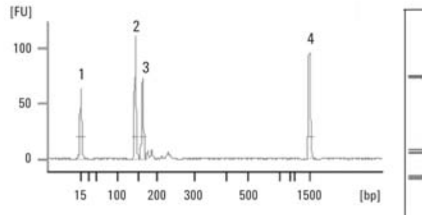
Strawberry sauce [30 ng DNA]



Overall results for sample 1: Strawberry sauce
Number of peaks found: 4
Peak table for sample 1: Strawberry sauce

Peak	Size [bp]	Conc. [ng/µL]	Molarity [nmol/L]	Observations
1	◀ 15	4.20	424.2	Lower marker
2	122	4.51	56.1	
3	134	0.59	6.7	
4	138	0.64	7.0	
5	282	1.69	9.1	
6	▶ 1,500	2.10	2.1	Upper marker

Strawberry sauce [30 ng DNA]



Overall results for sample 1: Strawberry sauce
Number of peaks found: 2
Peak table for sample 1: Strawberry sauce

Peak	Size [bp]	Conc. [ng/µL]	Molarity [nmol/L]	Observations
1	◀ 15	4.20	424.2	Lower marker
2	143	3.71	39.4	
3	161	3.01	28.2	
4	▶ 1,500	2.10	2.1	Upper marker

Kit: **DNA1000 kit**

Software Assay: **DNA 1000 assay**

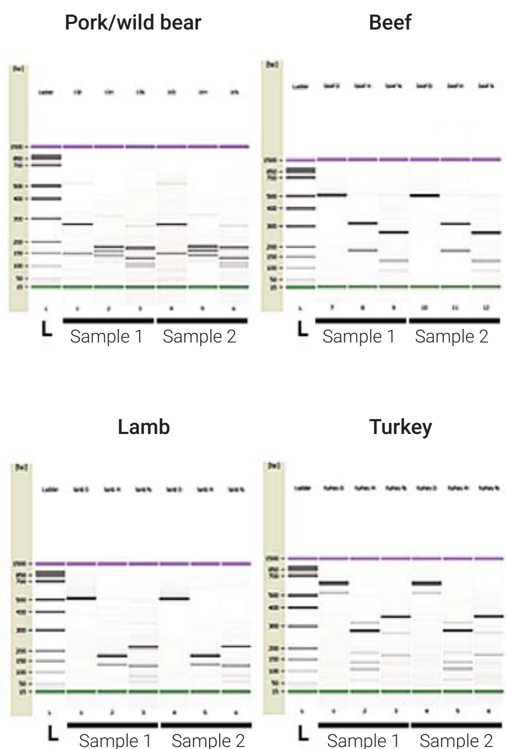
Abstract: Food authentication is important to help detect fraudulent replacement of expensive food ingredients, and to ensure correct ingredient content in prepacked foods. A PCR method followed by automated electrophoresis with the Agilent 2100 Bioanalyzer system and the DNA 1000 kit was used to distinguish strawberry and raspberry samples.

The figure shows the characteristic profiles obtained by the analysis of strawberry sauce through PCR with the microsatellite primer pairs Fvi11 and Fvi20. This method allows clear differentiation between samples that contain strawberry (*Fragaria*) and raspberry (*Rubus*) DNA and may prove useful in food authentication.

Application note: 5990-3327EN

Food analysis

Detection of non-fish species with PCR-RFLP



Kit: **DNA 1000 kit**

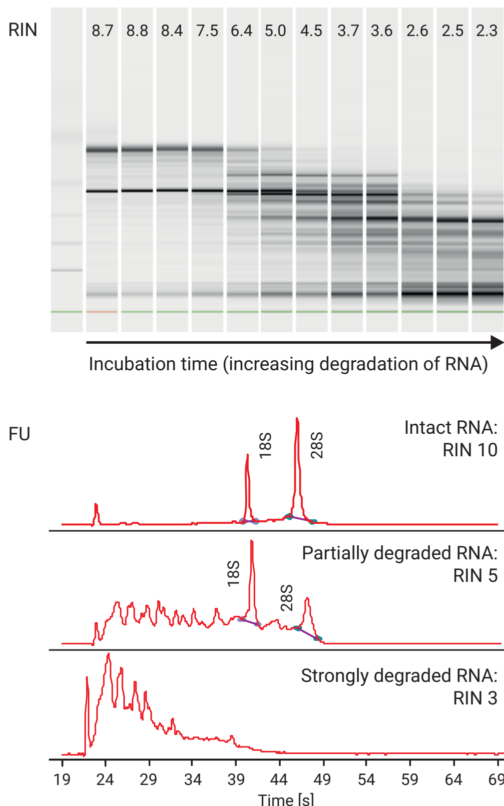
Software Assay: **DNA 1000 assay**

Abstract: A PCR-Restriction Fragment Length Polymorphism (RFLP) method was developed to identify fish species from processed foods using a cytochrome B PCR target sequence with analysis of the restriction pattern fragments on the Agilent 2100 Bioanalyzer system with DNA 1000 assay. Feasilbility for detection of non-fish species, especially mammalian or avian DNA originating from dairy products or meat, was tested. The assay was successfully used to differentiate pork, beef, and lamb. Pork and wild boar resulted in the same pattern. The figure shows the restriction digestion analysis on the Agilent 2100 Bioanalyzer system and the DNA 1000 assay. The gel-like images show the typical pattern obtained after digestion with three restriction enzymes (Dde I, Hae III, and Nla III) for two meat samples per species. Of avian samples, only turkey DNA resulted in easily identifiable patterns.

Application note: 5990-8452EN

Analysis of total RNA

Standardization of RNA quality control



Kit: **RNA 6000 Nano kit**

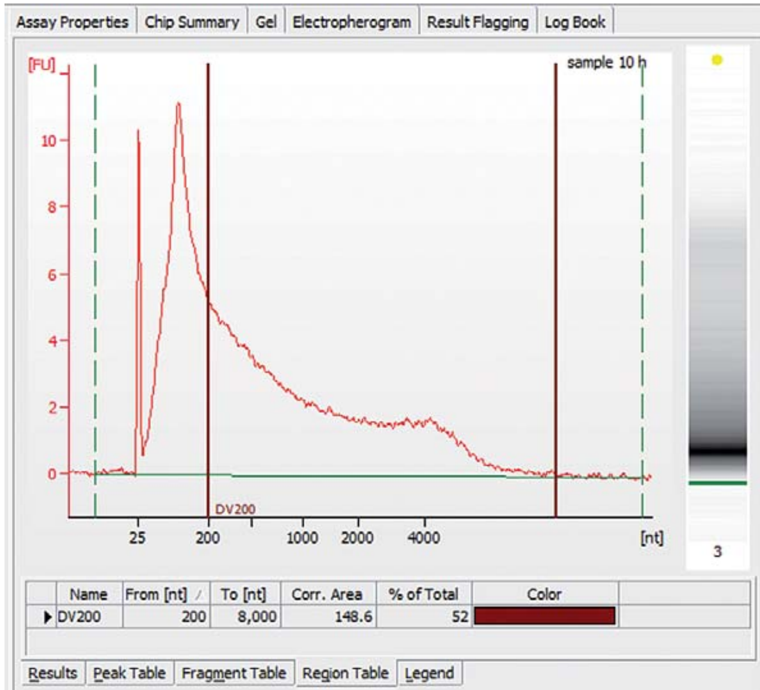
Software Assay: **Eukaryote Total RNA Nano assay**

Abstract: The RNA integrity number (RIN) is calculated by a dedicated software algorithm to assess the quality of RNA preparations. The RIN tool is a major step in the standardization of user-independent RNA evaluation and delivers more meaningful information than simple ratio calculations for ribosomal RNA peaks. It is not influenced by instrument, sample integration, and most importantly, concentration variability, thereby facilitating the comparison of samples and avoiding cost-intensive experiments with low quality RNA preparations. The RIN algorithm is based on a large collection of RNA data of various tissues and qualities. Furthermore, anomalies like genomic DNA contaminations are indicated with weighted error messages (critical/noncritical) to achieve maximum reliability.

Application note: 5989-1165EN

Analysis of total RNA

Quality control with DV200 evaluation of FFPE RNA samples



Kit: **RNA 6000 Nano and Pico kits**

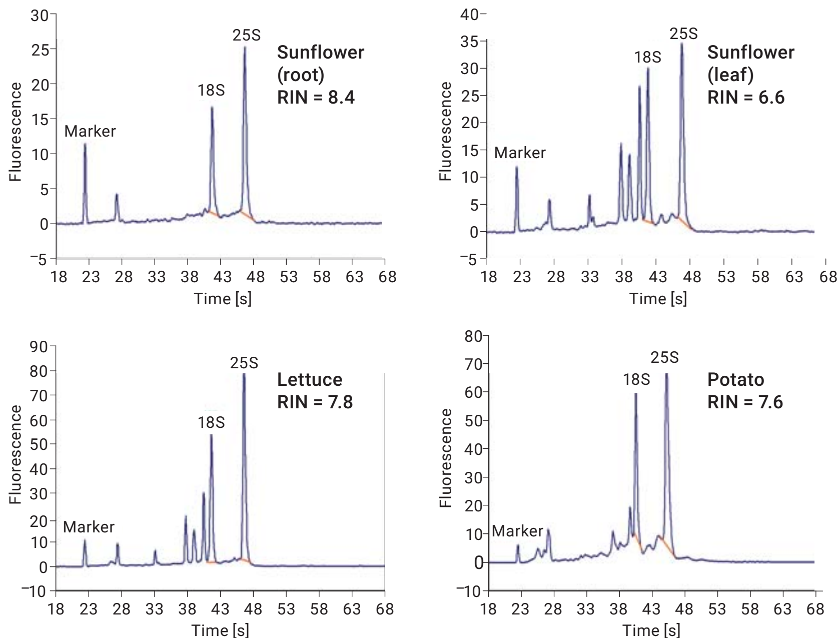
Software Assay: **DV200 RNA Nano and Pico assays**

Abstract: With the DV_{200} metric, degraded RNA samples extracted from FFPE tissue can be classified according to their size distribution. This Technical Overview describes the simplified evaluation of the DV_{200} using the DV_{200} RNA Nano and DV_{200} RNA Pico assays with the Agilent 2100 Bioanalyzer system. The assays can be used for re-analysis of existing data files as well as for new data acquisition. The DV_{200} RNA assays automatically define a region from 200 to 8000 nucleotides as shown in the figure. A corresponding DV_{200} is provided in the Region Table in the column % of Total. The DV_{200} results can be saved, exported, and displayed in reports. Samples with RIN <4 are flagged in different colors for easy assessment of the DV_{200} range.

Technical Overview: 5991-8287EN

Analysis of total RNA

Assessing integrity of plant RNA



Kit: **RNA 6000 Nano kit**

Software Assay: **Plant RNA 6000 Nano assay**

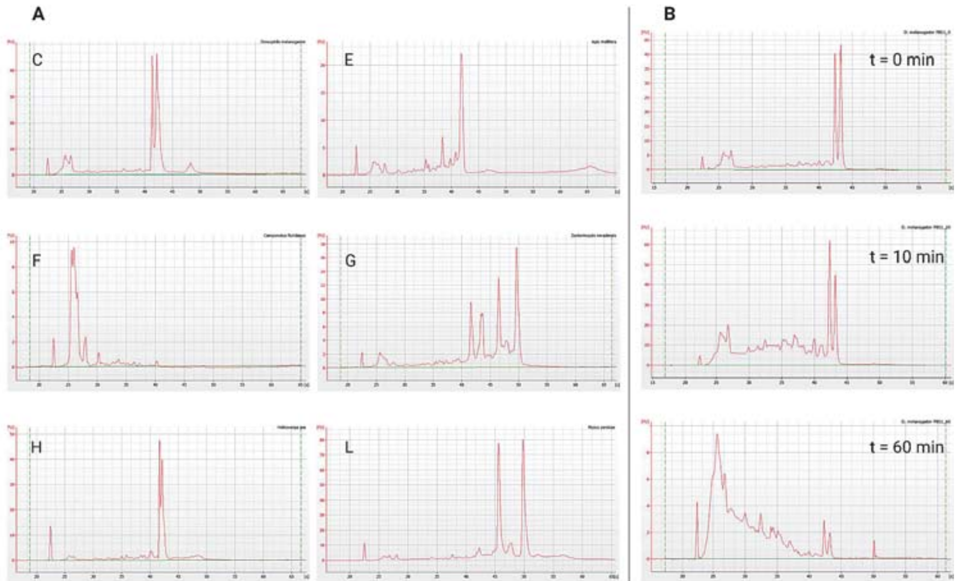
Abstract: High-quality RNA, free of genomic DNA, is a critical determinant for the success of many downstream techniques used in functional genomics, such as RT-PCR and microarray-based experiments. The dedicated Agilent 2100 Bioanalyzer plant RNA assay included in the 2100 Expert software (version B.02.07 or higher) allows rapid assessment of plant RNA integrity from multiple plant sources and differing degradation states with excellent precision. The figure shows the electrophoretic separation of different plant total RNA using the RNA 6000 Nano kit with the plant RNA assay. In all samples, the abundant 25S and 18S ribosomal RNA peaks are well resolved and automatically identified by the software. Compared to the root samples, the leaf and lettuce extracts exhibit additional fast migrating peaks corresponding to smaller chloroplast ribosomal RNAs showing that total RNA profiles can vary depending on species and tissue types.

The plant RNA assay and the RIN algorithm provide a convenient, user-independent assessment of total plant RNA integrity.

Application note: 5990-8850EN

Analysis of total RNA

Assessing integrity of insect RNA



Kit: **RNA 6000 Nano and Pico kits**

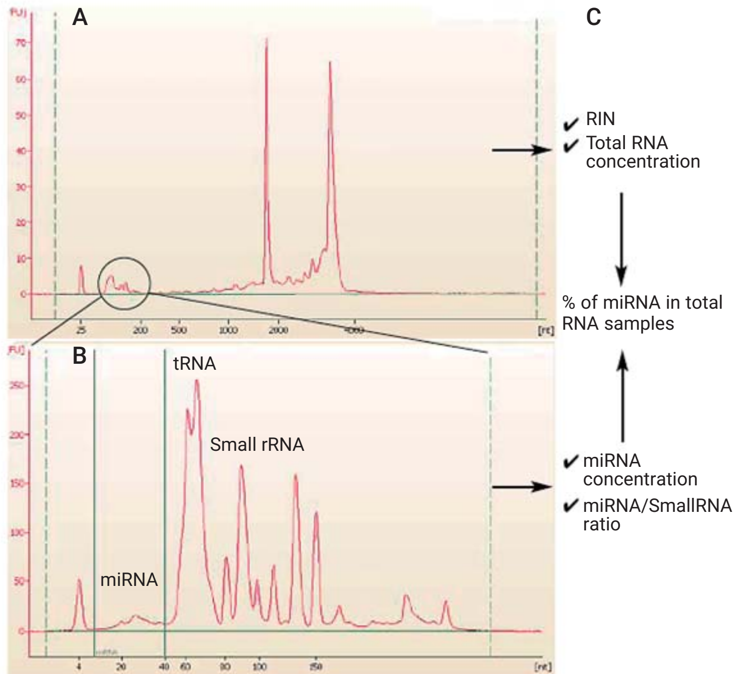
Software Assay: **Eukaryote total RNA Nano assay**

Abstract: This Application Note describes the complexity of insect RNA and the challenges associated with integrity determination using the 2100 Bioanalyzer system. Here, various extracts of insect species were analyzed for total RNA quality using the 2100 Bioanalyzer system and the obtained RNA profiles and RIN were compared. Figure A shows representative RNA profiles of *D. melanogaster (C), A. mellifera (E), C. floridanus (F), Z. nevadensis (G), H. zea (H),* and *M. persicae (L)*. The effect of degraded insect RNA on the outcome of quantitative RT-PCR experiments was evaluated with Figure B showing degradation of total RNA samples from *D. melanogaster* at room temperature for the indicated time. Even though the RIN, developed for eukaryotic total RNA, may not be practical for all insect species, the electropherograms provide suitable information to judge RNA integrity across insect species.

Application note: 5991-7903EN

Analysis of small RNAs

Analysis of miRNA content in total RNA samples



Kit: **Small RNA and RNA 6000 Nano kit**

Software Assay: **Small RNA and Eukaryote Total RNA Nano assay**

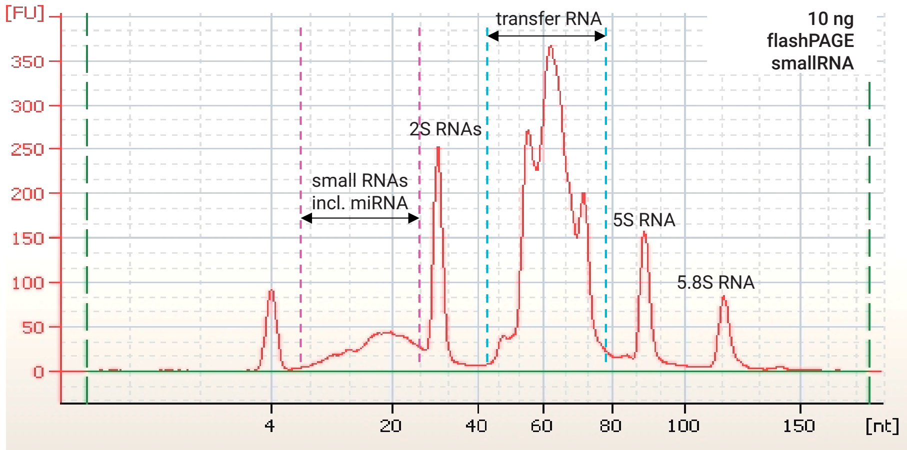
Abstract: Several total RNA samples containing small RNAs (including miRNAs, siRNA, and snRNA) were analyzed using the RNA 6000 Nano assay to determine concentration and quality, including RIN. All total RNA samples were then analyzed with the Small RNA assay to measure miRNA concentration. The relative amount of miRNA was manually calculated as a ratio of the concentration of miRNA in total RNA.

A) total RNA analyzed with the RNA 6000 Nano assay
B) small RNA analyzed with the Small RNA assay
C) miRNA analysis workflow

Application note: 5989-7870EN

Analysis of small RNAs

Analysis of small RNAs from *Drosophila* Schneider



Kit: **Small RNA kit**

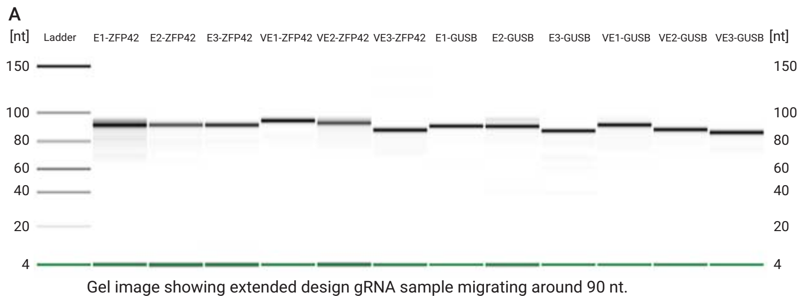
Software Assay: **Small RNA assay**

Abstract: Small RNA samples were prepared from Drosophila Schneider cells according to different protocols and were analyzed using polyacrylamide gel electrophoresis, northern blot, or the 2100 Bioanalyzer system. Only 10 ng of total RNA was run on the Small RNA kit. Two sharp peaks, corresponding to 5.8S and 5S RNA, were clearly resolved. Transfer RNA (tRNA) formed a third large peak. A small well-defined peak at 30 nucleotides indicated that 2S RNA could be detected with only 10 ng of total RNA. Therefore, the Small RNA kit was significantly more sensitive than a denaturing 15 % polyacrylamide gel stained with ethidium bromide or SYBR Green II, respectively. Hence, the Small RNA kit appeared to be the method of choice to assess small RNA species contained in a total RNA preparation.

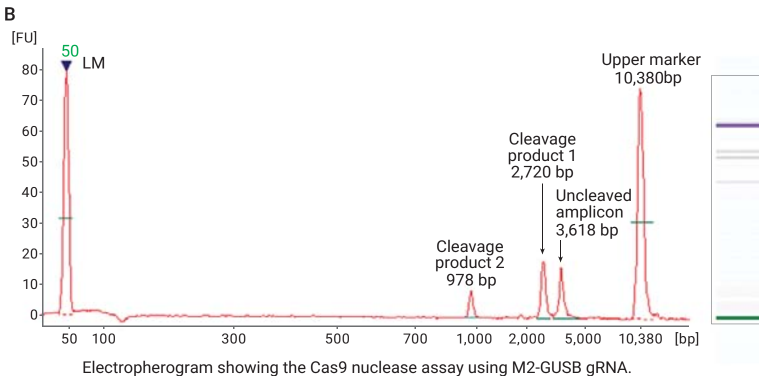
Application note: 5989-8539EN

Genome Editing

Guide RNA analysis for CRISPR-Cas9 Genome Editing



Gel image showing extended design gRNA sample migrating around 90 nt.



Electropherogram showing the Cas9 nuclease assay using M2-GUSB gRNA.

Kit: **Small RNA and DNA 7500 kits**

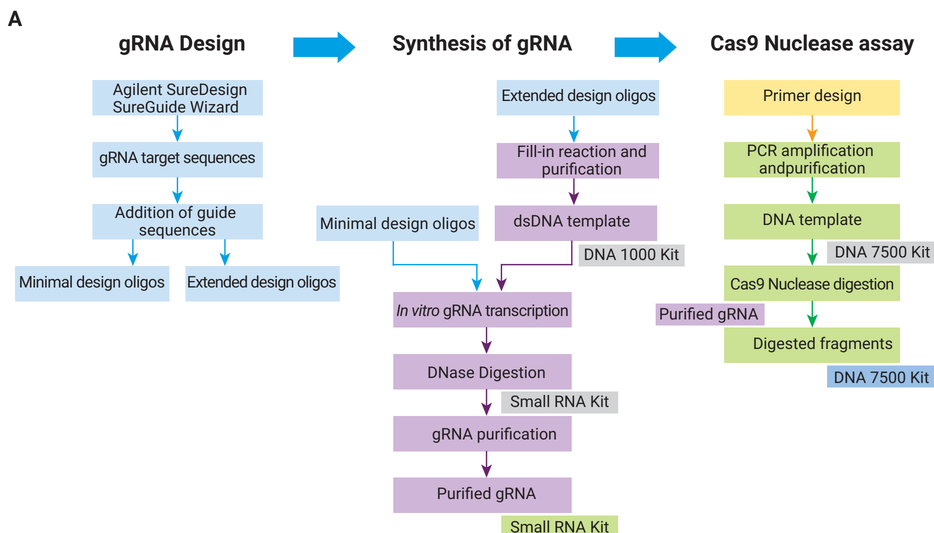
Software Assay: Small RNA and DNA 7500 assays

Abstract: This study demonstrates the seamless integration of Agilent solutions for guide RNA (gRNA) design, *in vitro* gRNA synthesis, and confirmation of gRNA-directed cleavage efficiency. Several gRNA sequences were designed using the Agilent SureDesign web-based application. The *in vitro* gRNA synthesis was carried out using the SureGuide gRNA synthesis kit. The transcribed and purified gRNAs were analyzed on the 2100 Bioanalyzer system with the Small RNA kit to assess the size, purity, and quantitation of each gRNA (Figure A) with a high level of accuracy and precision. The efficacy of the synthesized gRNAs was evaluated using the Agilent SureGuide Cas9 Nuclease kit. Successful gRNA-targeted cleavage of designed PCR amplicons by the Cas9 nuclease yields two fragments of expected sizes. The fragments were analyzed using the DNA 7500 kit to check for gRNA efficacy as presented in Figure B. The 2100 Expert software provides the areas of each peak for easy calculation of cleavage efficiency.

Application note: 5991-7557EN

Genome Editing

Protocol optimization for CRISPR-Cas9 workflows



Schematic diagram showing gRNA design, synthesis, and Cas9 assay workflow. The use of the 2100 Bioanalyzer sytem for quality control is shown as green, for efficacy assessment as blue and the grey fields indicate where it is suitable for optimizing the protocol.

Kits: **DNA 1000, Small RNA, and DNA 7500 kits**

Software Assay: **DNA 1000, Small RNA, and DNA 7500 assays**

Abstract: This study demonstrates the seamless integration of Agilent solutions for guide RNA (gRNA) design, *in vitro* gRNA synthesis, and confirmation of gRNA-directed cleavage efficiency with the overall workflow and quality checks highlighted in Figure A. In addition to quality control and efficacy assessment, the Agilent 2100 Bioanalyzer system can also be used to optimize the protocol at intermediate steps as indicated in Figure A. A few of these steps include:

– Extended design fill-in reaction – Verify the presence of successful fill-in products using the DNA 1000 kit and further optimize the reaction before *in vitro* transcription.

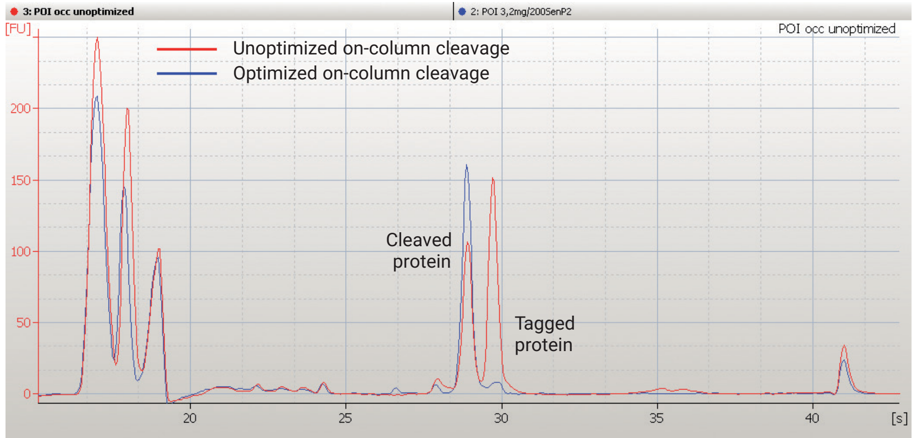
– Guide RNA purification – Check for successful *in vitro* transcription by running samples on the Small RNA kit before the final purification step. Optimize the input oligo concentration, yield, and incubation times.

– PCR amplification of the DNA template – gRNA specificity for the Cas9 nuclease assay requires PCR amplification of a designed amplicon. Confirm the correct size and yield with the DNA 7500 kit after PCR amplification, before use in the Cas9 nuclease assay.

Application note: 5991-7557EN

Protein purification

Optimization of on-column cleavage



Sample	Size [kDa]	Rel. Conc. [ng/µL]	% Total
Unoptimized on-column cleavage	71.9 81.9	310.2 414.7	38.3 51.3
Optimized on-column cleavage	70.9 83.2	993.0 11.4	78.0 0.9

Kit: **Protein 230 kit**

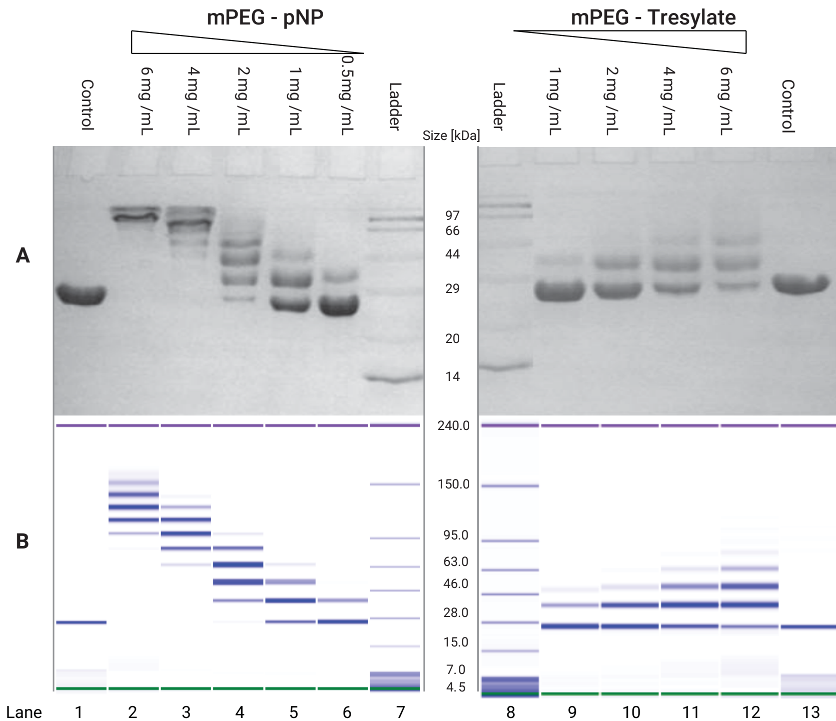
Software Assay: **Protein 230 assay**

Abstract: Monitoring the protein of interest (POI) during purification in a fast and reliable manner is fundamental. Here, protein purification of a His6-Sumo3-tagged DNA-binding protein was performed with Immobilized Metal Affinity Chromatography (IMAC). The POI was N-terminally fused to His6-Sumo3-tag, bound to Ni beads, and released from the tag and beads by treatment with His-tagged SenP2 protease. Although the protease was used in excess for on-column cleavage, most of the protein was still found to be fused to its tag. Therefore, the on-column cleavage step was optimized by determining the ideal ratio of protein-to-beads-to-protease by monitoring the percentage of cleaved protein versus its tagged precursor with the Protein 230 assay. The molecular weight difference of about 10 kDa is sufficient to achieve a baseline separation between the tagged and tagless protein. As illustrated in the blue electropherogram, almost all tagged protein can be cleaved under the optimized conditions.

Application note: 5990-6153EN

Analysis of modified proteins

Analysis of PEGylated proteins



Kit: **Protein 230 kit**

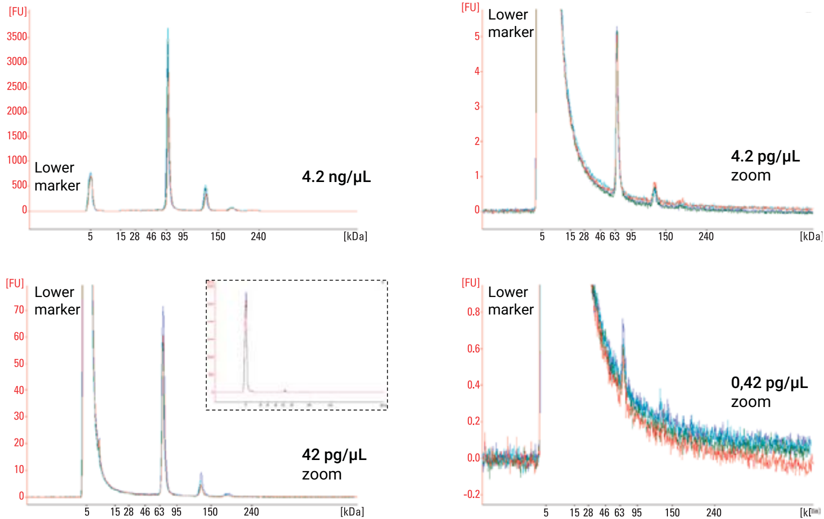
Software Assay: **Protein 230 assay**

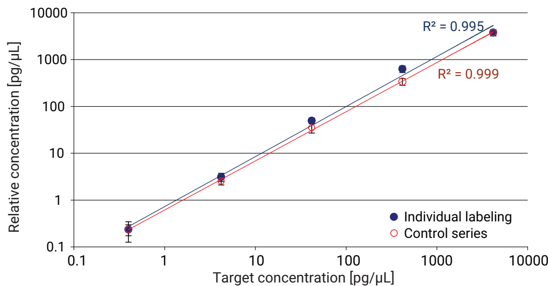
Abstract: Monitoring the degree of PEGylation is important to achieve a balance between retention of bio-activity, stability, and immunogenicity of the PEGylated species. SDS-PAGE is routinely used for PEGylation analysis, however, it cannot precisely identify the extent of the PEGylation. In addition, it can be tedious and time-consuming. The Agilent 2100 Bioanalyzer system was evaluated as an alternative to SDS-PAGE. A chimeric protein was subjected to PEGylation at 30 °C for 3 hours using different concentrations of two PEGylating reagents, mPEG-pNP (mol wt 5000) and mPEG-tresylate (mol wt 5000). Levels of PEGylation were monitored by SDS-PAGE (A) and the 2100 Bioanalyzer system (B). The profiles of PEGylated protein obtained with the Agilent 2100 Bioanalyzer system were superior and allowed differentiation between levels of PEGylation. The Agilent 2100 Bioanalyzer with Protein 230 assay is an easy-to-use tool that provides high resolution of PEGylated protein species, allowing efficient optimization of reaction conditions as well as fast and quantitative monitoring of production batches.

Application note: 5990-9593EN

High sensitivity protein detection

Detection of low abundant proteins





Kit: **High Sensitivity Protein 250 kit**

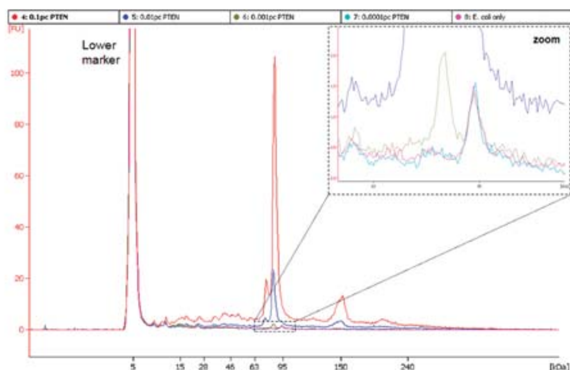
Software Assay: **High Sensitivity Protein 250 assay**

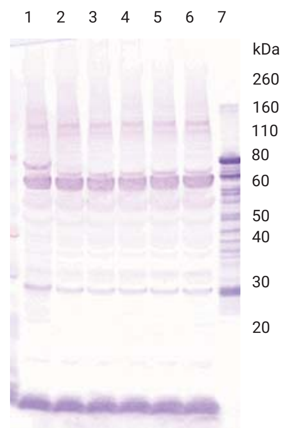
Abstract: For highest sensitivity, SDS-PAGE gels are commonly silver stained using a laborious procedure with low reproducibility and insufficient quantitation capabilities. The High Sensitivity Protein 250 kit is a superior alternative to silver staining protocols due to reproducible and fast staining, automated separation and data analysis. It overcomes the critical limitations of traditional silver staining of SDS-PAGE gels by providing high sensitivity and a linear dynamic quantitation range of four orders of magnitude combined with excellent reproducibility. The kit analyzes proteins from 10 to 250 kDa down to an on chip concentration of 1 pg/µL. It is based on the detection of fluorescently-labeled proteins that are separated by automated electrophoresis.

Application note: 5989-8940EN

High sensitivity protein detection

Highly specific and sensitive alternative to western blotting





1: 1 % PTEN
2: 0.1 % PTEN
3: 0.01 % PTEN
4: 0.001 % PTEN
5: 0.0001 % PTEN
6: *E. coli* only
7: PTEN only

Kit: **High Sensitivity Protein 250 kit**

Software Assay: **High Sensitivity Protein 250 assay**

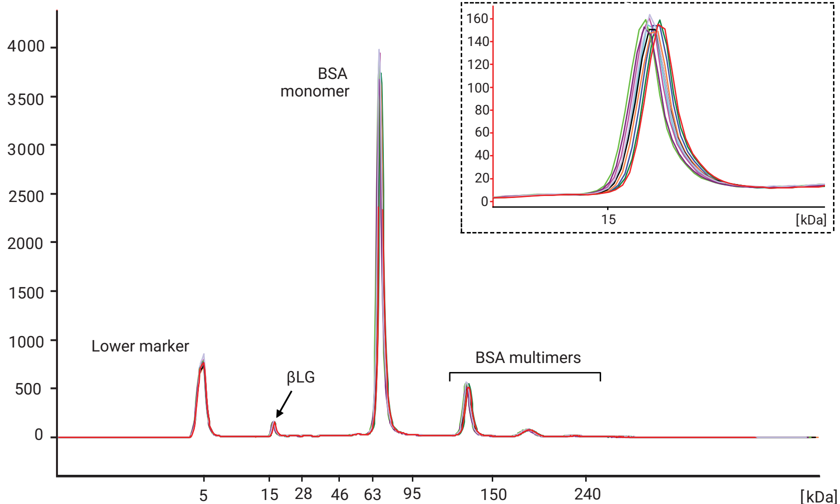
Abstract: A targeted protein analysis employing immunoprecipitation in combination with the High Sensitivity Protein 250 assay (IP/HSP250 method) was evaluated. Sensitivity and specificity of the IP/HSP250 method were investigated using *E. coli* lysate spiked with GST tagged phosphatase and tensin homolog (PTEN). Samples were first fluorescently labeled, then immunoprecipitated, and finally the complexes formed were directly eluted from magnetic Protein A beads and analyzed with the High Sensitivity Protein 250 assay. Electropherograms of samples with 0.1 % to 0.0001 % PTEN and a negative control are shown. The zoom shows the main peak of 0.001 % PTEN. The limit of detection was determined to be 0.001 % or 100 pg PTEN in 10 µg *E. coli* lysate. For comparison, western blots were performed. The PTEN blots showed a high nonspecific background in all lanes, due to the secondary antibody. A specific band was observed only at 1 % or 100 ng PTEN in 10 µg *E. coli* lysate.

Thus, the IP/HSP250 method showed both higher sensitivity and specificity than the western blot, resulting in a 1,000 fold lower limit of detection for PTEN.

Application note: 5990-4097EN

High sensitivity protein detection

Quantitation of low protein abundance with an internal standard



Peak		Size [kDa]	Rel. Conc. [pg/ µL]	% Total
bLG	AVERAGE	16.9	184	3.3
	%CV	1.4	2.3	1.5
BSA monomer	AVERAGE	68.7	4191	75.7
	%CV	0.8	3.1	0.3

Kit: **High Sensitivity Protein 250 kit**

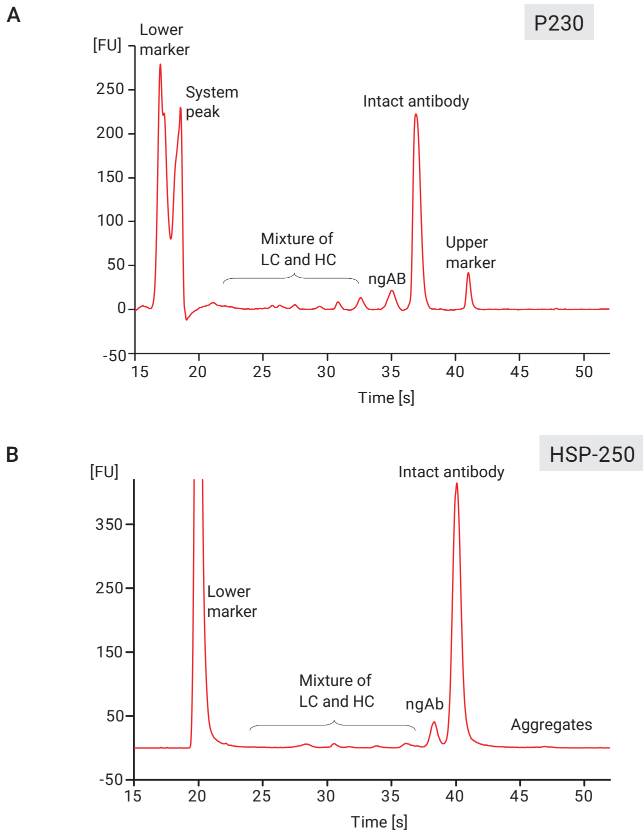
Software Assay: **High Sensitivity Protein 250 assay**

Abstract: The High Sensitivity Protein 250 assay is based on the detection of fluorescently-labeled proteins that are separated by automated electrophoresis. The quantitation and sizing of sample peaks is done relative to a ladder as an external standard on the same chip, in contrast to the Agilent Protein 80 and Protein 230 assays where the quantitation is based on internal standards. With the High Sensitivity Protein 250 kit, it is also possible to do quantitation based on the addition of a suitable internal standard. In this example, a small standard protein, ß-Lactoglobulin (ßLG, 18.4 kDa), was used. ßLG was spiked into a BSA solution before the labeling reaction and the analysis was performed according to the standard protocol. The electropherogram shows an overlay of 10 runs and a zoom on the ßLG peak in the insert. The quantitation data for the ßLG and BSA monomer is summarized in the table. Quantitation reproducibility is improved using an internal standard, because well-to-well variations for sample injection and matrix influences during staining are excluded.

Application note: 5989-8941EN

Antibody analysis

Analysis of IgG2 under nonreducing conditions



Kit: **Protein 230 and High Sensitivity Protein 250 kits**

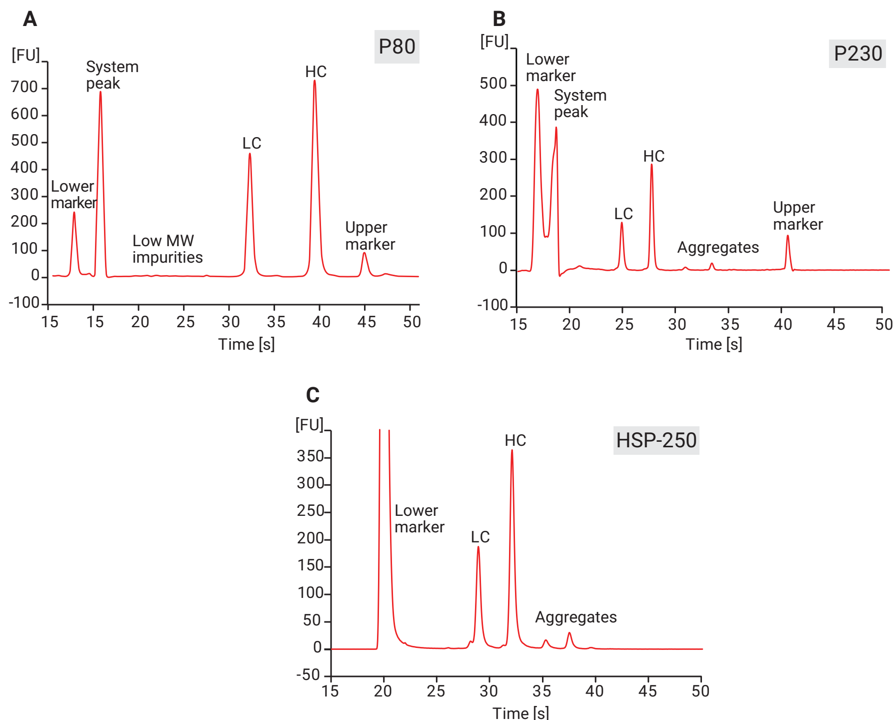
Software Assay: **Protein 230 and High Sensitivity Protein 250 assays**

Abstract: Human myeloma IgG2 was analyzed with the 2100 Bioanalyzer system and the Protein 230 (P230) and High Sensitivity Protein 250 (HSP-250) assay under nonreducing conditions. One representative electropherogram per assay is shown. The intact IgG2 antibody is detected at 156.6 kDa, which is in close agreement with its theoretical molecular mass of about 150 kDa. A unique feature of the HSP-250 assay is the size and concentration measurement beyond the size range of the ladder, that is, 250 kDa. Therefore, high molecular weight aggregates or impurities above 250 kDa are sized and quantified as well. The P230 and HSP-250 assays clearly resolve light chain (LC), heavy chain (HC) and a mixture of LC and HC peaks including the nonglycosylated form of IgG2 (ngAb).

Application note: 5990-5283EN

Antibody analysis

IgG2 analysis under reducing conditions



Kit: **Protein 80, 230 and High Sensitivity Protein 250 kits**

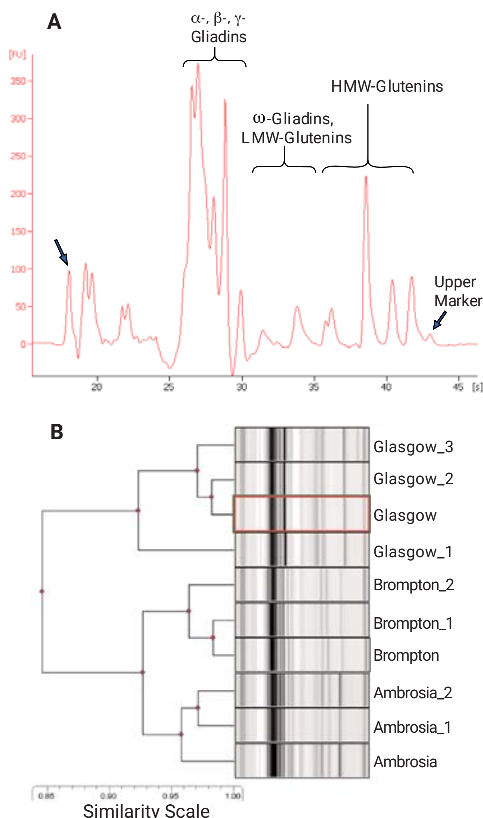
Software Assay: **Protein 80, 230 and High Sensitivity Protein 250 assays**

Abstract: Human myeloma IgG2 was analyzed with the 2100 Bioanalyzer system and the Protein 80 (P80), the Protein 230 (P230), and the High Sensitivity Protein 250 (HSP-250) assay in the presence of dithiothreitol (DTT) as reducing agent. One representative electropherogram per assay is shown. Under reducing conditions, the IgG2 light chain (LC) and heavy chain (HC) are well resolved with all three available protein assays. Aggregates of higher molecular weight are observed with the P230 and HSP-250 assays whereas the P80 assay resolves low molecular weight (mol wt) impurities associated with the IgG2 sample.

Application note: 5990-5283EN

Food analysis

Rapid wheat varietal identification



Kit: **Protein 230 kit**

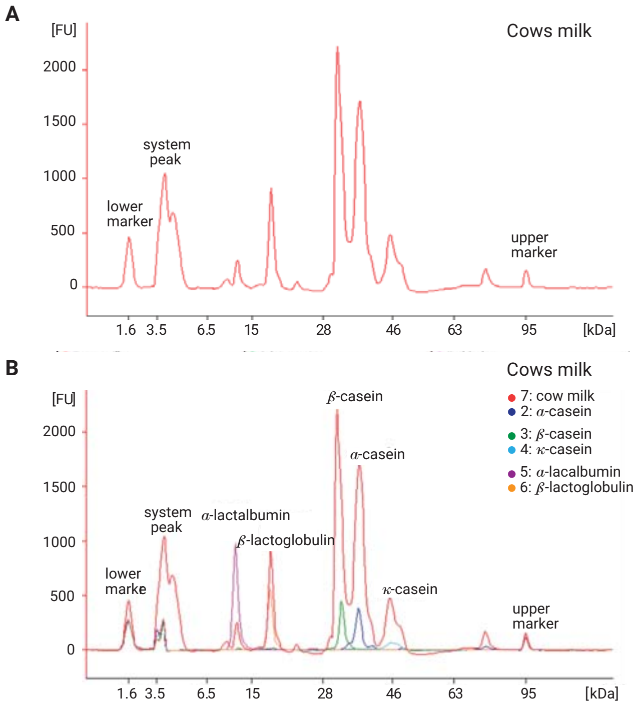
Software Assay: **Protein 230 assay**

Abstract: Total wheat proteins (including high molecular weight glutenins, HMW) were extracted from individual grains. The extract samples were separated on the 2100 Bioanalyzer system. The Protein 230 assay produced well-resolved protein profiles, suitable for varietal discrimination (A). Electropherogram profiles were processed using the Phoretix 1D Advanced and 1D Database (Nonlinear Dynamics) software for pattern-matching purposes. Replicates of three different wheat varieties can be correctly grouped in a dendrogram (B). The study has demonstrated that using the 2100 Bioanalyzer system with the Phoretix system offers a standardized, objective method for rapid varietal discrimination. The ease-of-use and short analysis times of less than one hour from sampling to 2100 Bioanalyzer system result makes it most suitable for mill intake use.

Application note: 5989-7735EN

Food analysis

Protein analysis in milk



Kit: **Protein 80 kit**

Software Assay: **Protein 80 assay**

Abstract: Qualitative and quantitative information about the main protein species in milk is an important quality criterion. Cow, goat, and sheep milk were analyzed by automated electrophoresis with the Agilent Protein 80 kit and the Agilent 2100 Bioanalyzer system. The figure shows the analysis of commercially available cows milk. The five major milk proteins were analyzed separately and overlaid with the milk electropherogram to identify the milk proteins. The electropherograms allow an easy and reliable comparison of different samples. Furthermore, milk from different species could be distinguished based on their protein pattern which facilitates incoming inspection in routine labs. The Agilent 2100 Bioanalyzer system and the Protein 80 kit provide a fast, reproducible, and robust qualitative and quantitative method for milk analysis.

Application note: 5990-8125EN

Agilent 4200 TapeStation system

Learn more at:
www.agilent.com/genomics/tapestation

Building on the success of the 2100 Bioanalyzer system, the Agilent 4200 TapeStation system offers 96-well plate compatibility combined with scalable throughput. These features make it an ideal solution for quality control of biological samples in next-generation sequencing, microarray, and qPCR workflows in high throughput applications. The system offers walk away operation with fully automated sample processing. Any sample number from 1 up to 96 samples can be analyzed at constant cost-per-sample.

- **DNA size and quantity**
  Quality control of NGS libraries and genomic DNA samples
- **RNA quality check with RIN^{e}**
  Integrity and quantitation of euk. and prok. total RNA samples

The 4200 TapeStation system uses credit card-sized, ScreenTape consumables that are available for DNA, and RNA and applications. Sample analysis has never been so easy – simply load the 4200 TapeStation instrument with ScreenTape consumable, loading tips, and your samples in 2 x 8 tube strips or a 96 well plate. An integrated pipette transfers the samples onto the ScreenTape device. After automated electrophoresis and imaging, results are available in as little as 1 minute per sample. The ready-to-use ScreenTape technology enables ultimate flexibility for switching between assays as well as sample preparation. With sensitive detection and zero carryover, you get the utmost confidence in your results, ensuring your downstream workflow is a complete success.

The 4200 TapeStation software includes functionality for instrument control and data analysis. It is intuitive and presents the automatically analyzed data as familiar gel image and electropherogram for each sample. With a few mouse-clicks the software generates customized reports or enables data export in various different formats.

Additional benefits

- Unused lanes on the ScreenTape consumable can be used at a later point of time.
- Achieve user-independent results with minimal manual intervention and excellent reproducibility for sizing, concentration, and integrity assessment.
- Requires only 1-2 µL of DNA or RNA sample per run, even for high sensitivity analysis.
- Rely on the integrity standards for total RNA (RNA Integrity Number equivalent, RIN^e) and genomic DNA (DNA Integrity Number, DIN).
- Carryover is eliminated as the ScreenTape consumable analyses each sample in a separate lane with individual loading tips.
- Sample evaporation is avoided by covering the 96-well plate with pierceable foil.
- Save valuable bench space in your lab with a compact system offering the complete sample QC solution.

The D1000 ScreenTape assay facilitates the separation and analysis of DNA fragments, fragmented DNA, and NGS libraries from 35 - 1,000 bp. Choose between the D1000 and the High Sensitivity D1000 ScreenTape assay depending on the sensitivity requirements of your application. Both assays allow accurate and reliable sizing and quantitation. The High Sensitivity ScreenTape assay requires only 2 µL sample input and has a sensitivity of 5 pg/µL per fragment. ScreenTape consumables, sample buffer, as well as ladder are available as standalone products.

The D5000 ScreenTape assay allows for the separation and analysis of DNA fragments from 100 - 5,000 bp, ideal for larger sized NGS libraries, PCR products, or restriction digests. Depending on the expected sample concentration, it is possible to choose between the D5000 ScreenTape and the High Sensitivity D5000 ScreenTape application.

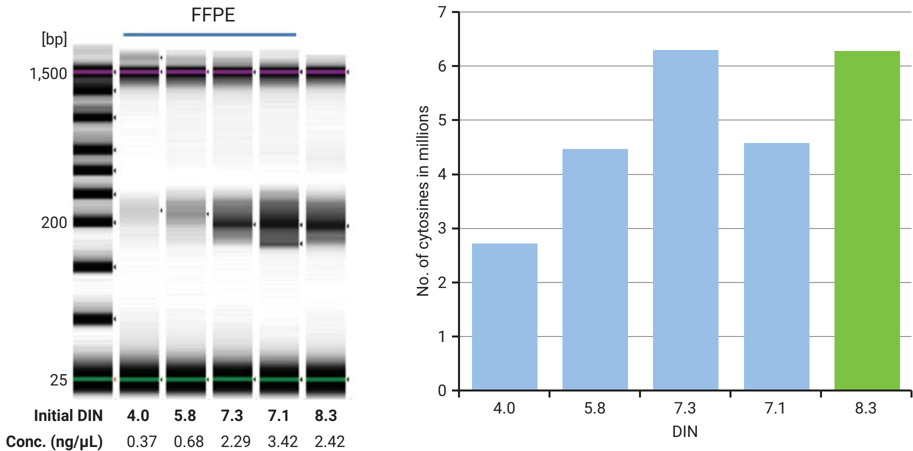
The Genomic DNA ScreenTape assay was developed for the separation and analysis of genomic DNA (gDNA) from 200 to greater than 60,000 bp. It provides accurate quantitation and sizing data as well as an automated numerical assessment of gDNA quality based on the DNA integrity number (DIN) and is therefore the ideal QC tool for next generation sequencing (NGS) and array comparative genomic hybridization (aCGH) workflows. The DIN algorithm is included in the 4200 TapeStation software, and provides a numerical assessment of the DNA sample by assigning a numerical score from 1 to 10. A high DIN indicates highly intact gDNA, and a low DIN a strongly degraded gDNA sample. The Genomic DNA ScreenTape assay allows sample quantitation in the range of 10-100 ng/μL. The functional range for the DNA integrity number is specified between 5 and 300 ng/μL.

The RNA ScreenTape assay provides efficient and reliable RNA analysis for RNA characterization and quality assessment. The RNA integrity number equivalent (RIN^e) delivers an instant and objective evaluation of RNA degradation for eukaryotic and prokaryotic total RNA samples. The RIN^e number is directly comparable to the widely accepted and highly cited RNA Integrity Number (RIN) of the Agilent 2100 Bioanalyzer system. A high RIN^e indicates highly intact RNA, and a low RIN^e a strongly degraded RNA sample. In addition, the RNA ScreenTape assay returns quantitative information as well as the ribosomal ratio of eukaryotic and prokaryotic samples.

Choose between the RNA ScreenTape and the High Sensitivity RNA ScreenTape assay depending on the requirements of your application. The High Sensitivity RNA ScreenTape assay allows a detection of RNA samples down to 200 pg/μL with a sample volume need of only 2 μL.

Next generation sequencing

Impact of gDNA Integrity on the Outcome of DNA Methylation Studies



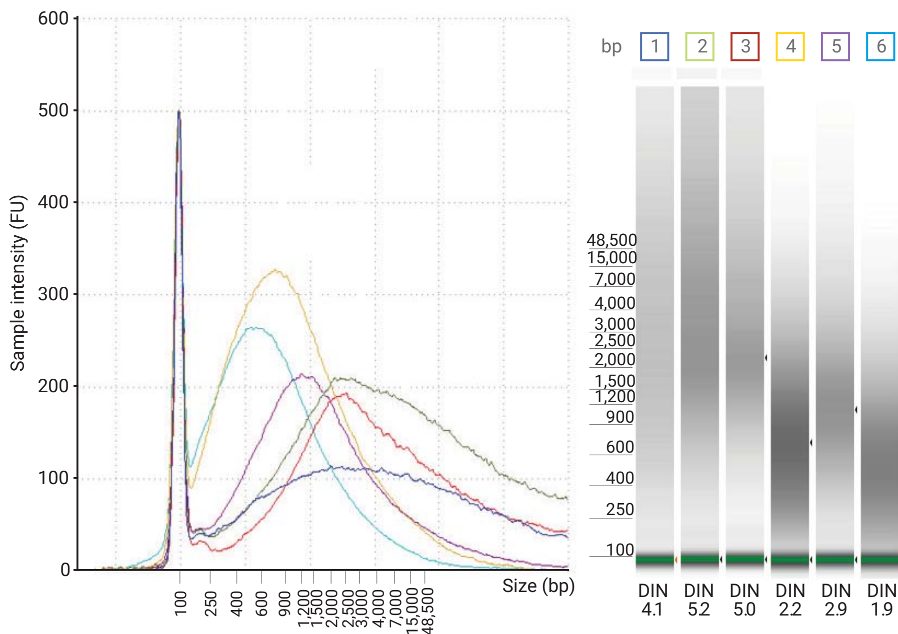
Assay: **Genomic DNA ScreenTape Assay**

Abstract: Human brain tissue from a single donor was used for the genome-wide DNA methylation analysis. Genomic DNA was extracted from fresh frozen tissue and used as control. Four tissue samples were subjected to FFPE using varying lengths of paraffin treatment times to generate varying levels of DNA degradation. The gDNA integrity of this starting material was determined using the Genomic DNA ScreenTape assay and the DNA Integrity Number (DIN). To investigate the correlation between DIN and the quality of the sequencing results, the number of covered CpG sites was determined and compared to the DIN of the initial gDNA samples. The bar chart demonstrates that the total number of covered CpG sites varies, depending on initial DIN value of the gDNA samples. The DIN can be used as quality criteria to determine how to handle individual gDNA samples for downstream workflows, and to ensure successful DNA methylation analysis.

Application note: 5991-6427EN

Next generation sequencing

Analysis of DNA from FFPE tissue



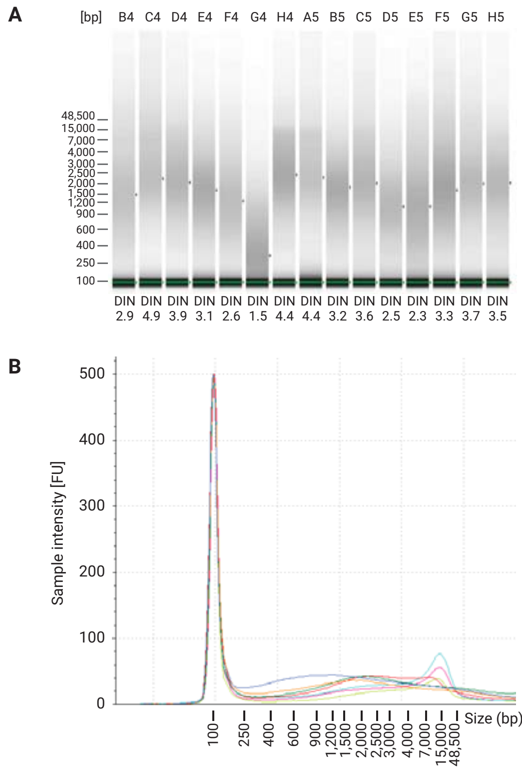
Assay: **Genomic DNA ScreenTape Assay**

Abstract: Sequencing of genomic DNA (gDNA) from FFPE archived tissue can be challenging, as the obtained material is often of variable quality. 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. The DIN correlates with key sequencing quality metrics and, thus, presents an integrity threshold for the processing of FFPE samples.

Application note: 5991-5360EN

Next generation sequencing

Quality Control of DNA from FFPE tumor samples



Assay: **Genomic DNA ScreenTape Assay**

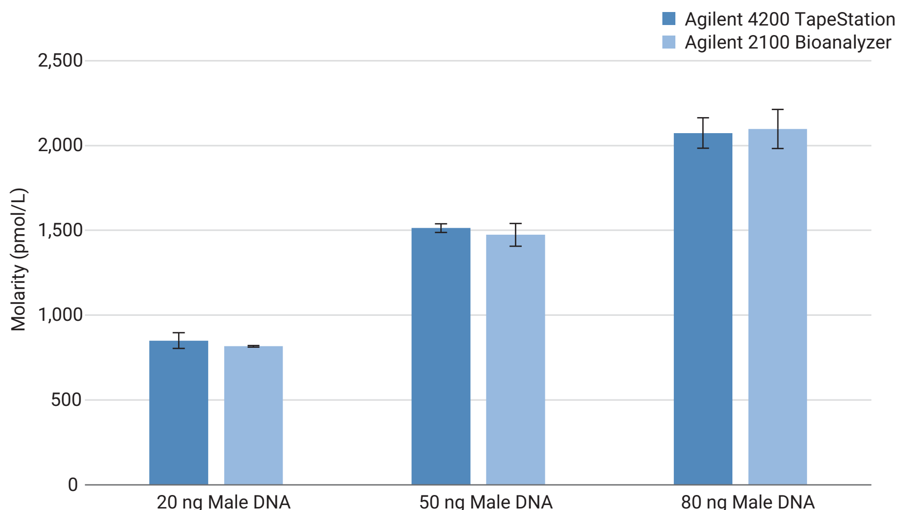
Abstract: To determine if samples were suitable for NGS library preparation, a quality control (QC) was performed at the beginning for a batch of 88 genomic DNA (gDNA) samples from FFPE tumor tissue by the German Cancer Research Center (DKFZ) High Throughput Sequencing Unit. This initial QC includes quantitation and analysis with a 4200 TapeStation system and the Genomic DNA assay to determine DNA quality based on the DNA integrity number (DIN).

The figure shows a representative subset of the data collected on the 4200 TapeStation system with the Genomic DNA ScreenTape assay. gDNA samples extracted from FPPE material are often of low DNA integrity, but can still be sufficiently intact for whole exome library preparation and successful sequencing.

Application note: 5991-7615EN

Next generation sequencing

Quantitation of amplified SureSelect^{QXT} whole genome libraries



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

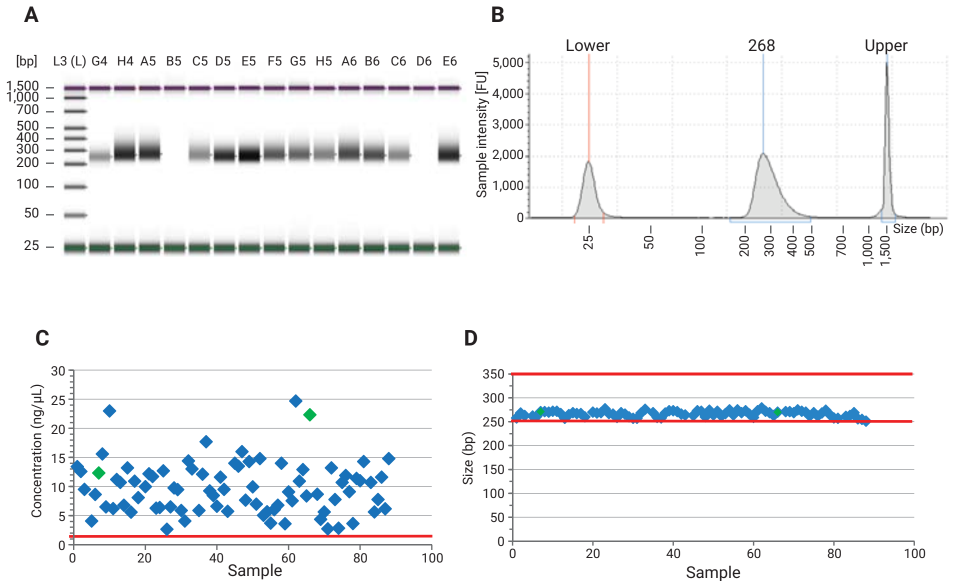
Assay: **High Sensitivity D5000 ScreenTape Assay**

Abstract: For multiplex sequencing, SureSelect^{QXT} whole genome libraries are combined such that each index-tagged sample is present in equimolar amounts in the pool. The 4200 TapeStation and 2100 Bioanalyzer systems provide molarity quantitation data along with the sizing information in the region table of the software. For each library generated by various genomic DNA (gDNA) input amounts, the molarity was plotted in a graph comparing both systems as presented in the above figure. The data summarized in the table demonstrates that sizing and quantitation of amplified libraries with the High Sensitivity D5000 ScreenTape assay match the results of the High Sensitivity DNA assay of the 2100 Bioanalyzer system and results can be considered as equivalent.

Application note: 5991-8191EN

Next generation sequencing

Quality Control of final NGS libraries



Assay: **D1000 ScreenTape Assay**

Abstract: The 4200 TapeStation system was used for the 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.

A) Gel view of 15 samples, lane B5 and D6 show negative controls. B) Example of an electropherogram of one sample. The Agilent D1000 assay includes a lower and upper marker. C) The distribution of the concentration for all 80 samples plus eight controls. The two positive controls are shown as green symbols. The red lines indicate the recommended concentration threshold (2 ng/µL). D) The size distribution of all 80 samples plus eight controls. The two positive controls are shown as green symbols. The red lines indicate the recommend size range (250 to 350 bp).

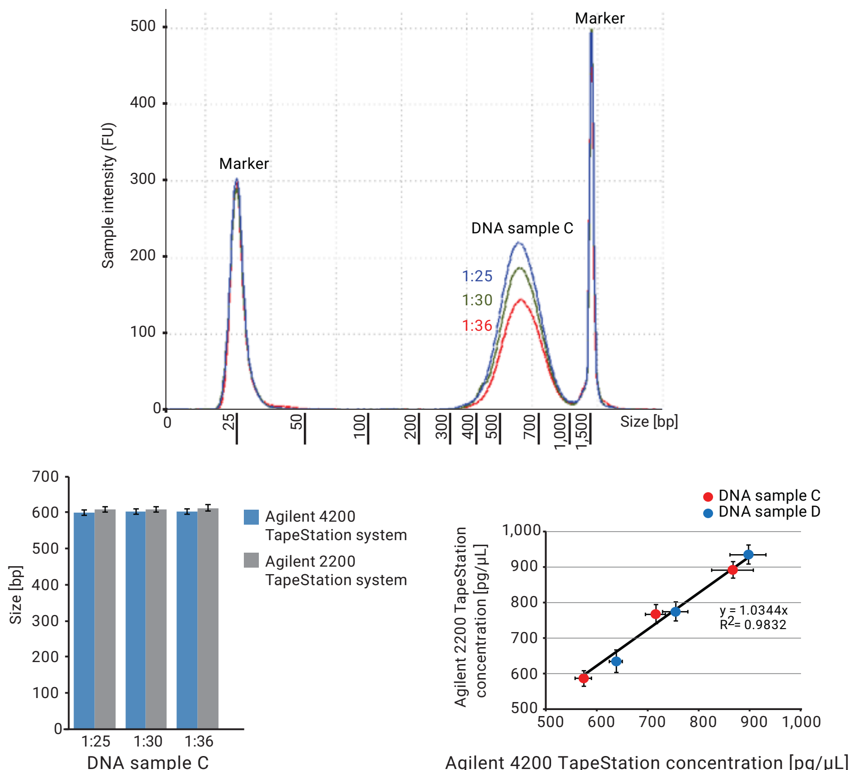
The analysis of the final NGS libraries with the 4200 TapeStation system confirmed successful DNA library preparation for all 80 samples and the six positive control samples.

Application note: 5991-7615EN

Next generation sequencing

Analysis of NGS Libraries

Equivalence of the 4200 TapeStation and 2200 TapeStation systems



Assay: **D1000 and High Sensitivity D1000 ScreenTape Assay**

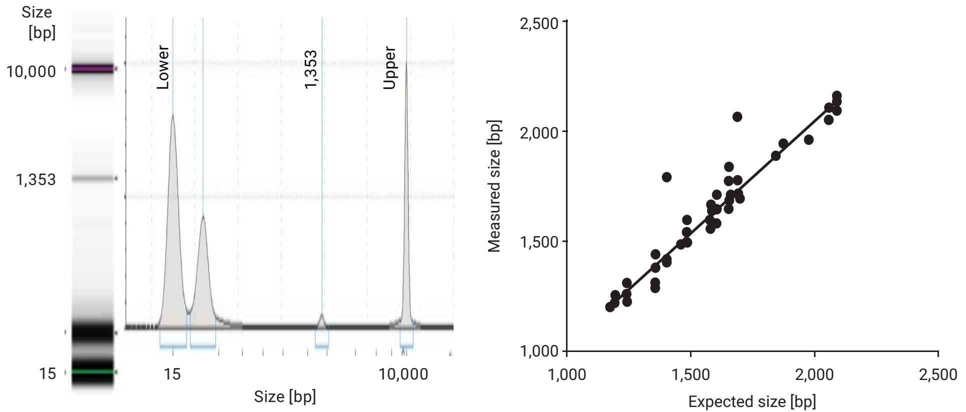
Abstract: Quality control of NGS libraries is the key for the success of any sequencing run. The D1000 and High Sensitivity ScreenTape assays can be used for quality control, providing DNA sizing, and quantitation. The bar chart shows the sizing results of the same sample set analyzed with the High Sensitivity D1000 ScreenTape assay and the 2200 and 4200 TapeStation platform. 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.

Application note: 5991-6892EN

PCR product analysis

Quality Control of Single Cell DNA Samples



Assay: **D5000 and High Sensitivity D5000 ScreenTape Assay**

Abstract: With current methodologies, both the genome and the whole cell transcript content can be measured and characterized from a single cell. These analyses require amplification of single cell starting material for detection. Most-commonly polymerase chain reaction (PCR) is used for this purpose. To limit the errors introduced by PCR, specific high-fidelity enzymes are required. In addition, the number of PCR cycles should be kept to a minimum. The Agilent D5000 and Agilent High Sensitivity D5000 ScreenTape assay can be used for quality control screening of limited-cycle nested PCR amplification of multiple low-abundance genomic targets from single cells for polymorphic analysis by NGS.

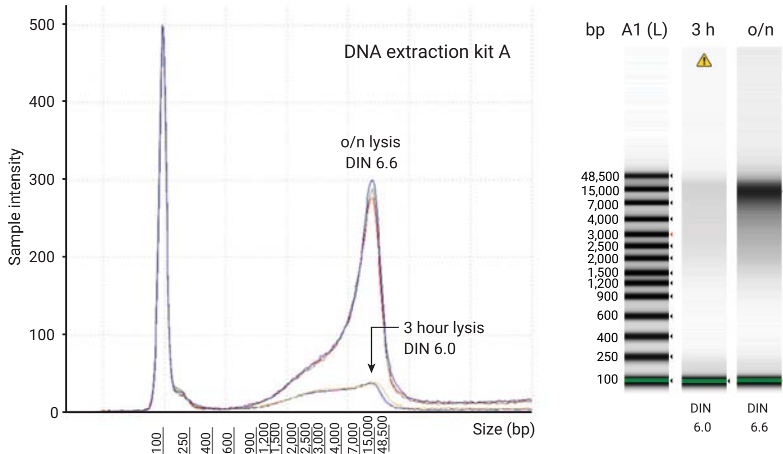
The figure shows a characteristic gel image and electropherogram of one of the amplicons analyzed using the D5000 ScreenTape assay which was nondetectable by ethidium bromide stained agarose gels. The right plot displays the correlation between expected amplicon size and amplicon size measured with the Agilent D5000 ScreenTape assay.

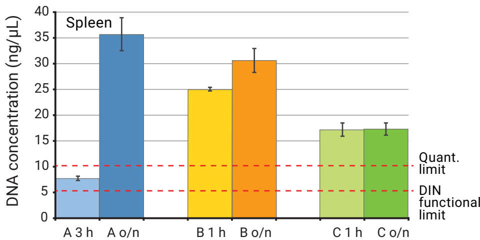
Both TapeStation assays provide unique sizing accuracy and sensitivity, making the detection of amplicons from low-abundance targets from single cells much easier as compared to traditional agarose gel-electrophoresis.

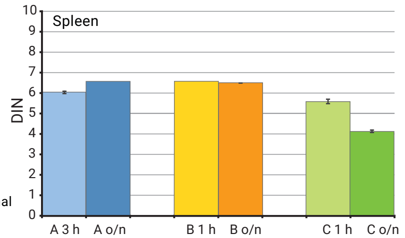
Application note: 5991-5259EN

Genomic DNA Analysis

Optimization of gDNA Extraction from FFPE tissue







Assay: **Genomic DNA ScreenTape Assay**

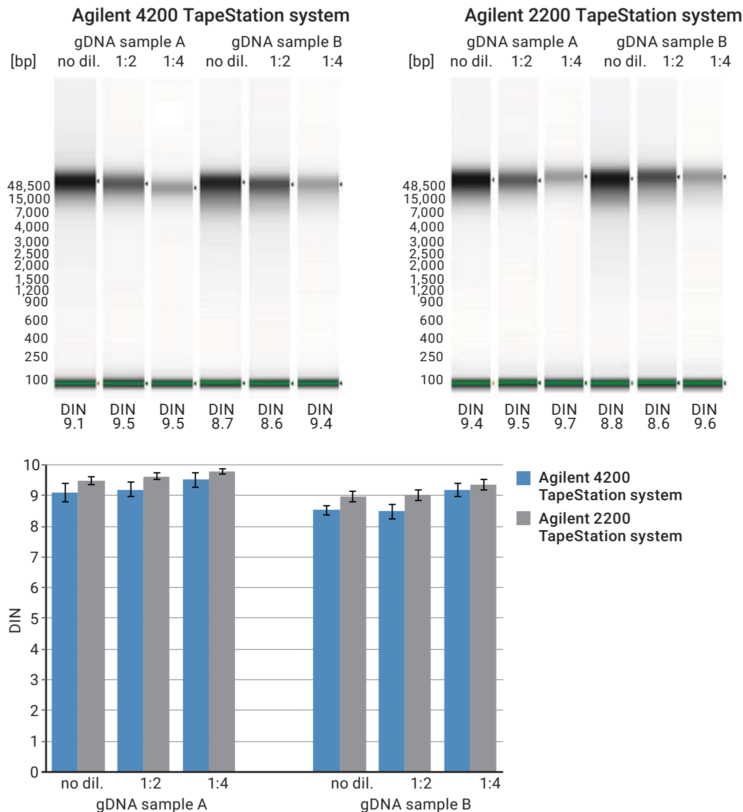
Abstract: One of the most widely used methods for tissue preservation and archiving is the preparation of formalin-fixed paraffin-embedded (FFPE) samples. These FFPE tissue archives represent a valuable source for retrospective studies of gene expression and mutation analysis. However, DNA extraction from FFPE samples has been proven to be challenging. To compare the effect of DNA extraction method and the influence of tissue type on the quantity and quality of the extracted DNA, different mouse tissues isolated by three commercially available DNA extraction kits (vendor A, B and C) were investigated. The electropherogram and the gel image shown in the figure above summarize the results obtained for the DNA extracted from mouse spleen FFPE tissue for one extraction kit.

The Genomic DNA ScreenTape assay and the DNA Integrity Number (DIN) provide a valuable and reliable tool for quality control of DNA extraction, with quantitation determination and automated sample integrity assessment. The DIN is presented automatically, and, thus, does not require a subjective integrity estimation or approximation based on user experience.

Application note: 5991-5246EN

Genomic DNA Analysis

Equivalence of the 4200 TapeStation and 2200 TapeStation systems



Assay: **Genomic DNA ScreenTape Assay**

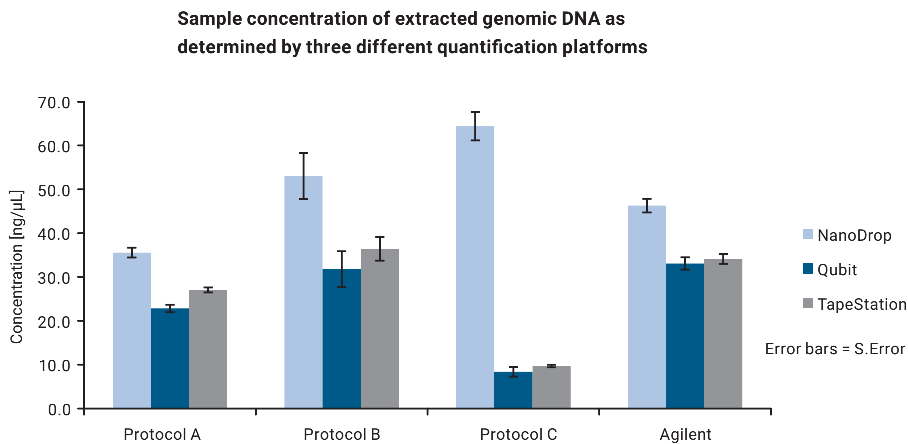
Abstract: To evaluate the difference between the 2200 TapeStation system and the 4200 TapeStation system, two commercially available gDNA samples were analyzed at three different concentrations using the Genomic DNA ScreenTape assay. The DNA Integrity Number (DIN) is automatically determined and directly 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 directly 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 TapeStation systems is 4 % for all tested samples and concentrations.

Application note: 5991-6892EN

Genomic DNA Analysis

Quantitation of isolated genomic DNA (gDNA) samples



Assay: **Genomic DNA ScreenTape Assay**

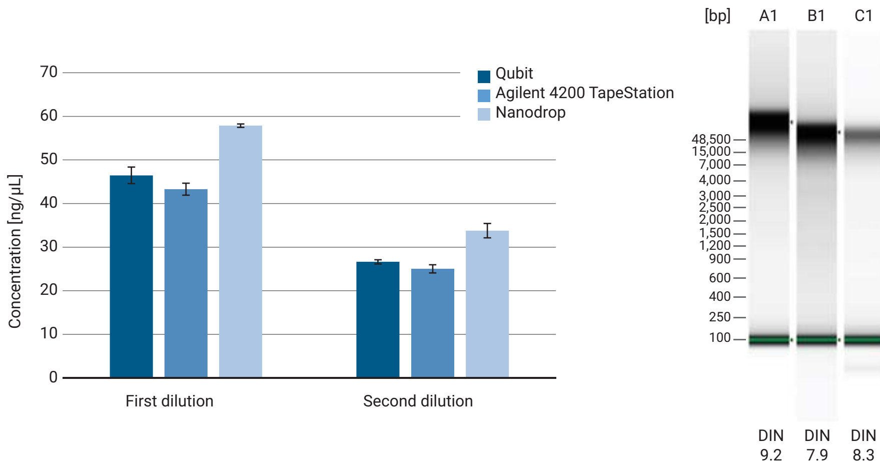
Abstract: The Genomic DNA ScreenTape assay in combination with the TapeStation system allows the automatic analysis of up to 96 genomic DNA samples. With the DNA Integrity number (DIN) the assay provides automatic determination of sample integrity as well as sample quantitation. In the above example, genomic DNA from HEK293 cells was isolated with extraction kits from different suppliers. Samples were analyzed as triplicates and the quantitation data was collected for each kit. The samples were also quantified using the Qubit dsDNA broad range assay and NanoDrop spectrophotometer to compare results. The mean values obtained from the Qubit, NanoDrop, and the Genomic DNA ScreenTape assay were plotted, compared, and shown above.

The data shows that the ScreenTape and Qubit analysis provide very similar results with a minimum average difference of 1 % and a maximum average difference of 5 %. The CV percentages are similar across all three platforms for each kit illustrating differences between each triplicate extracted sample rather than any performance variances in the platforms.

Application note: 5991-1797EN

Genomic DNA Analysis

Quantity and Integrity Analysis of genomic DNA for Next Generation Sequencing



Assay: **Genomic DNA ScreenTape Assay**

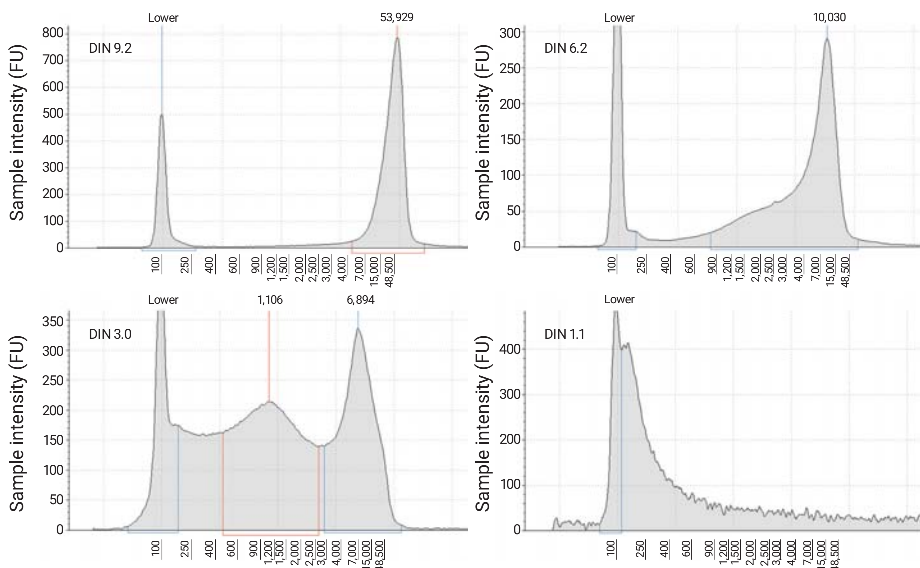
Abstract: The Agilent SureSelect^{QXT} WGS protocol requires high-quality DNA samples for optimal performance and precise quantitation of the gDNA starting material. Serial quantitation was carried out using the Qubit instrument with the dsDNA BR Assay in accordance to the protocol. The same samples were analyzed on the Genomic DNA ScreenTape assay with the 4200 TapeStation system and the NanoDrop with six replicates on each instrument. Data from the 4200 TapeStation system, Qubit, and NanoDrop are presented in the above figure, showing the applicability of the Genomic DNA ScreenTape assay in quantitating genomic DNA starting material. As shown in the above bar chart, quantitation results of the 4200 TapeStation and the Qubit instrument are in good agreement. 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 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.

Application note: 5991-8191EN

Genomic DNA Analysis

Integrity analysis of Genomic DNA from bacterial sources



Assay: **Genomic DNA ScreenTape Assay**

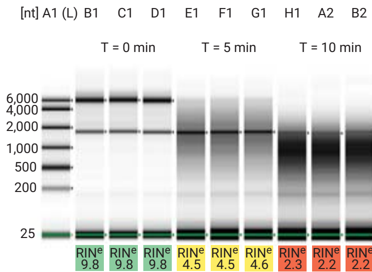
Abstract: The DNA Integrity Number (DIN) was developed to facilitate the quality control of isolated genomic DNA (gDNA) samples. The DIN software algorithm allows for the classification of total DNA based on a numbering system from 1 to 10, with 1 being the most degraded and 10 being the most intact. The DIN facilitates the interpretation of electropherograms, allows for the comparison of samples, and ensures the repeatability of experiments and quantitation of high-quality gDNA moving into e.g. next generation sequencing library construction.

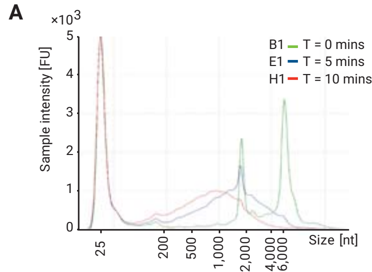
The above electropherograms illustrate the variability of the analyzed genomic DNA samples isolated from bacteria. DIN numbers range from 9.2 down to 1.1. In earlier studies, it was determined that samples with a DIN of > 7 were acceptable to progress into the next step of library construction. In the example above, 3 out of 4 samples did not pass the QC criteria for the downstream application.

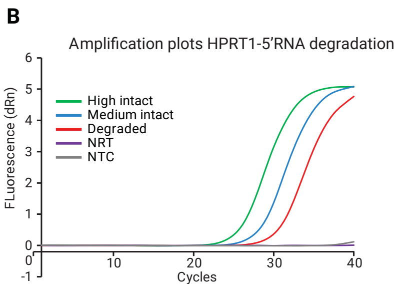
Application note: 5991-5442EN

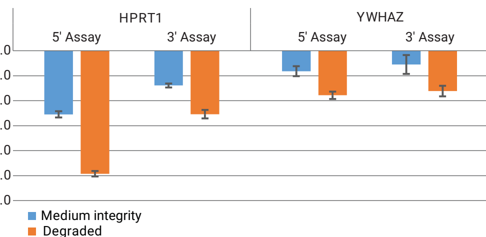
Gene Expression Analysis

Impact of RNA degradation on qPCR experiments









Assay: **RNA ScreenTape Assay**

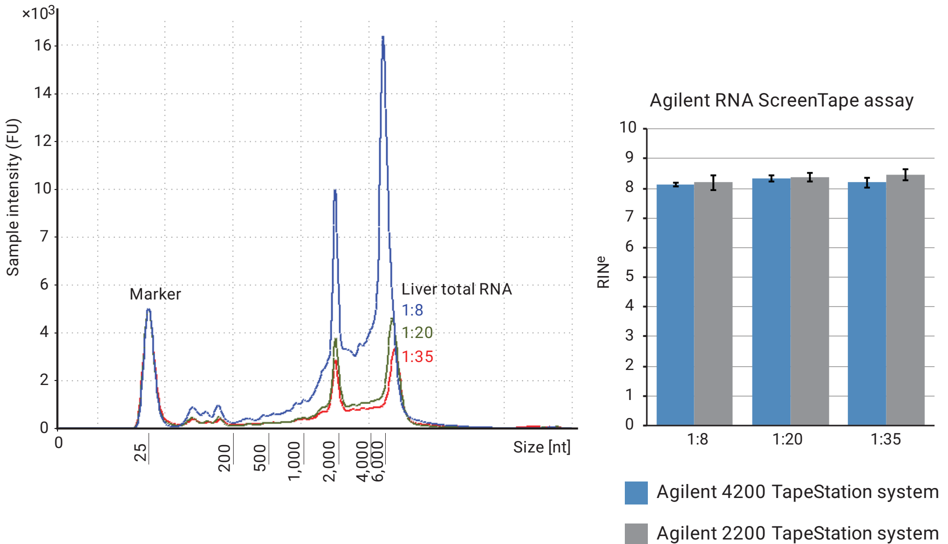
Abstract: The importance of using only high integrity RNA samples for real-time quantitative PCR (RT-qPCR) experiments as recommended by MIQE (Minimum Information for Publication of Quantitative Real-Time PCR Experiments) guidelines was shown by carrying out RT-qPCR amplification using heat-degraded RNA as a template. The differentially heat-degraded RNA and untreated RNA were analyzed using the 2200 TapeStation and 2100 Bioanalyzer systems and designated as high, medium, and low integrity based on the RNA integrity number equivalent (RIN^e) The gel image as well as the electropherogram trace overlay shows RNA with three different levels of degradation.

qPCR amplifications were carried out using different integrities of total RNA. The amplification plots are shown in the lower left figure. The C_q was determined for all primer pairs. The C_q value of the untreated and highly intact RNA sample was taken as a reference. The relative C_q difference of the treated RNA templates was calculated, and is presented in the lower right figure. These results show that differential degradation is specific to the respective gene products and that the RNA ScreenTape assay can be reliably used in determining the integrity of total RNA samples. The TapeStation system provides an ideal system to fit into any qPCR workflow in accordance with the MIQE guidelines.

Application note: 5991-4971EN

Analysis of total RNA

Equivalence of the 4200 TapeStation and 2200 TapeStation system RNA integrity number



Assay: **RNA and High Sensitivity RNA ScreenTape Assay**

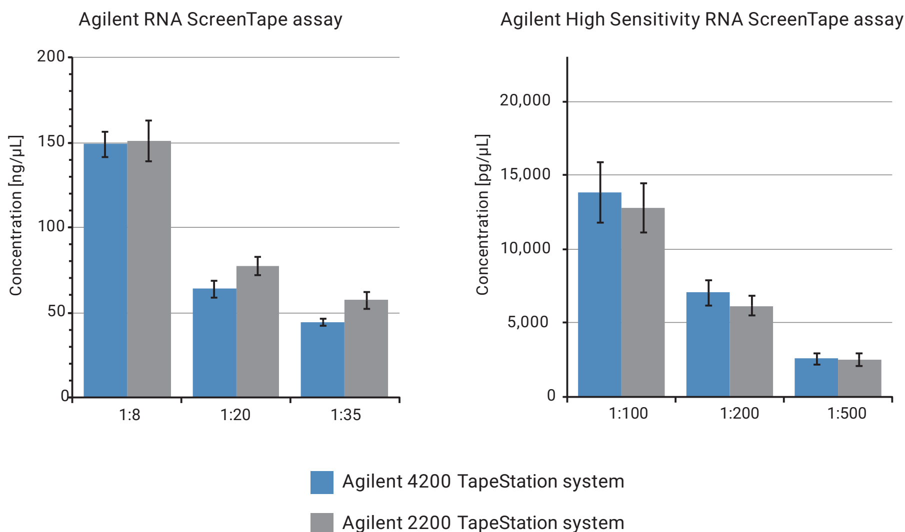
Abstract: As RNA is highly sensitive to degradation, quality control is essential, and usually includes RNA integrity analysis and quantitation. The above electropherogram overlay represents different dilutions of liver total RNA analyzed on the 4200 TapeStation system and the RNA ScreenTape assay.

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 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 hands-on-time, allowing the analysis of 96 samples without manual intervention. This is a primary requirement specifically in a high throughput environment.

Application note: 5991-6892EN

Analysis of total RNA

Equivalence of the 4200 TapeStation and 2200 TapeStation system quantitation



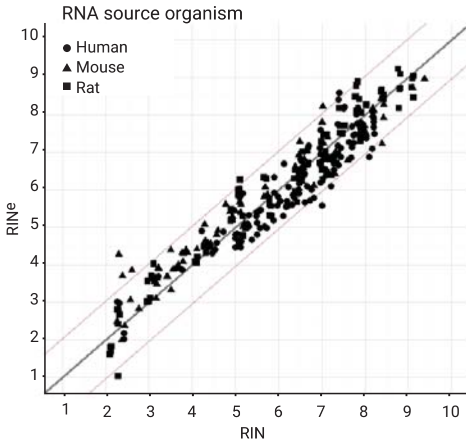
Assay: **RNA and High Sensitivity RNA ScreenTape Assay**

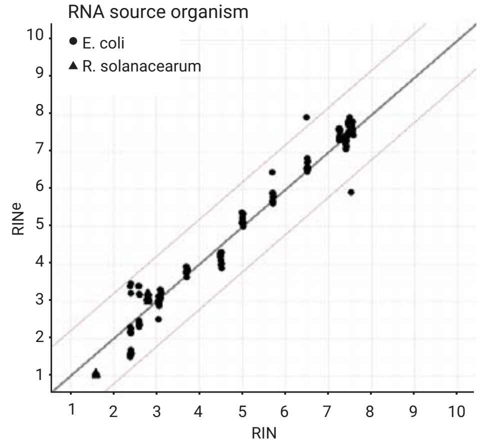
Abstract: Liver total RNA of various concentrations was quantified with the Agilent RNA ScreenTape assay and the Agilent High Sensitivity RNA ScreenTape assay using the Agilent 4200 TapeStation system and the Agilent 2200 TapeStation system (n=15). The data demonstrates that the total RNA quantitation with both TapeStation systems is comparable for the 2 assays. The total RNA quantitation precision with the 4200 TapeStation system was below 15 % CV for the RNA and High Sensitivity RNA ScreenTape assays.

Application note: 5991-6892EN

Analysis of total RNA

Comparison of the RNA integrity analysis on 2200 TapeStation with 2100 Bioanalyzer system





Assay: **RNA ScreenTape Assay**

Abstract: Agilent pioneered the reliable and robust assessment of total RNA quality with the introduction of the RNA Integrity Number (RIN) for the 2100 Bioanalyzer system. Despite differences in the technologies between the two systems, the 2200 TapeStation system provides the same quality assessment with the RNA integrity number equivalent (RIN^e).

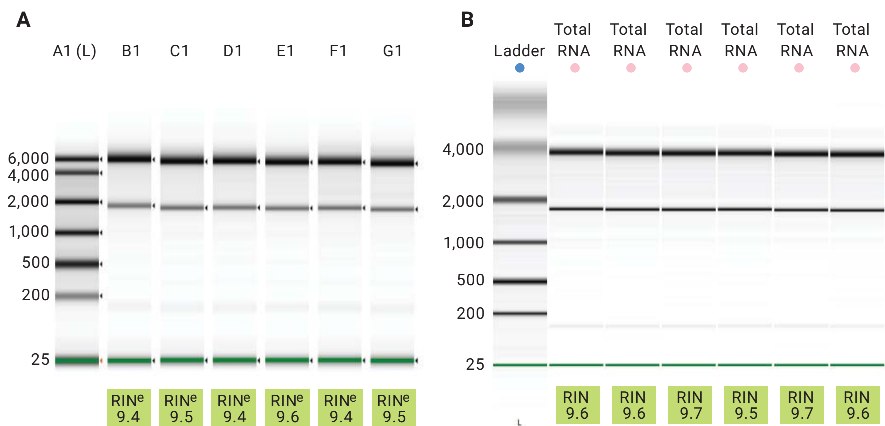
To demonstrate that RIN and RIN^e are directly comparable, various RNA samples were analyzed on both the 2100 Bioanalyzer and the 2200 TapeStation systems. RIN^e and RIN, values obtained for eukaryotic total RNA were plotted against each other (left figure). The median error calculated versus the 2100 Bioanalyzer system was a 0.4 RIN unit difference, and a standard deviation of 0.28 RIN units.

A similar approach was taken to compare the obtained RIN^e and RIN values from prokaryotic total RNA (right figure). Similar results were obtained for the prokaryotic RNA with a median error of 0.2 RIN units and a standard deviation over dilution series of 0.16 RIN units.

Technical Overview: 5991-3426EN

Analysis of total RNA

Equivalence of RNA analysis on 2200 TapeStation and 2100 Bioanalyzer systems



	Quantitation (ng/µL)		Integrity (RINe/RIN)	
System	Agilent 2200 TapeStation	Agilent 2100 Bioanalyzer	Agilent 2200 TapeStation	Agilent 2100 Bioanalyzer
Average	128.8	116.5	9.5	9.56
CV %	2.8	7.4	0.9	0.8

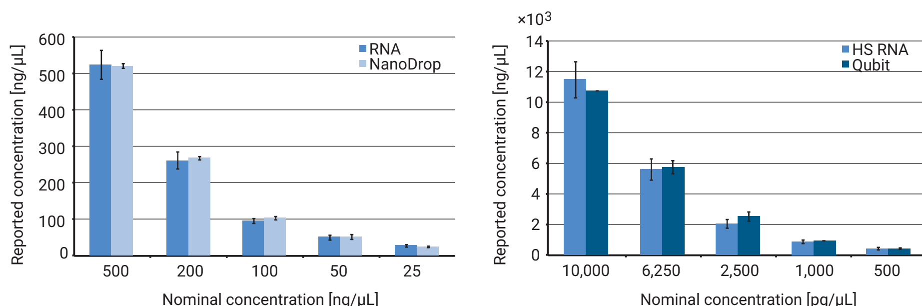
Assay: **RNA ScreenTape Assay**

Abstract: Quality control of total RNA to determine its integrity is essential to ensure that high quality starting material is used for e.g. RNA-Seq library preparation. The total RNA isolated from the HEK293 cell lines was analyzed using the RNA ScreenTape and RNA 6000 Nano assays on the 2200 TapeStation and 2100 Bioanalyzer systems, respectively. The SureSelect protocol indicates that a RNA integrity number (RIN) of 8 or above should be used for RNA library preparation. Gel-like images generated by the analysis software from both instruments are shown in the above figure together with calculated RINe and RIN values with an average of 9.5. The table summarizes the RNA integrity and quantity metrics obtained from both instruments, and shows that the 2200 TapeStation system is highly comparable to the 2100 Bioanalyzer system for quantitation and analyzing the integrity of total RNA.

Application note: 5991-4116EN

RNA Quantitation

RNA Quantitation with the TapeStation system in comparison with spectrophotometric and fluorescence based measurements



Assay: **RNA and High Sensitivity RNA ScreenTape Assay**

Abstract: To determine the quantitation accuracy and reproducibility of the RNA ScreenTape assay, rat kidney total RNA samples were prepared at five different nominal concentrations ranging from 25 to 500 ng/µL. The same set of samples was analyzed with the RNA ScreenTape assay on the 2200 TapeStation system and the NanoDrop 2000 system by four different analysts (left figure). Both quantitation methods, the RNA ScreenTape assay and the NanoDrop 2000 system, yielded comparable results for the tested concentrations.

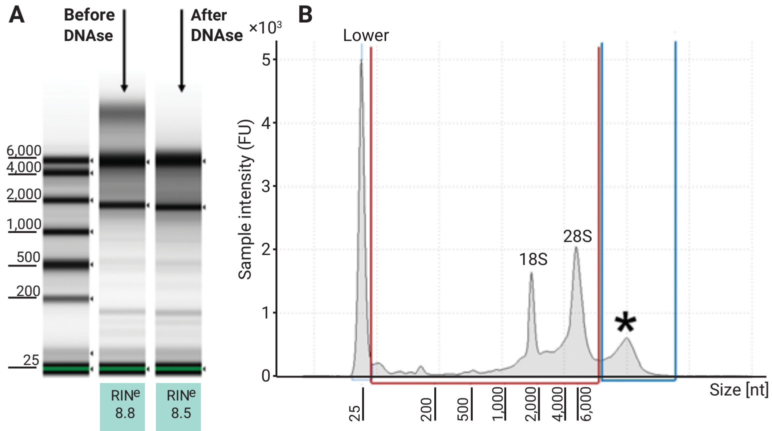
Similarly, the quantitation accuracy and reproducibility of the High Sensitivity RNA ScreenTape assay was tested. Rat kidney total RNA samples were prepared at five different nominal concentrations ranging from 500 to 10,000 pg/µL. The samples were analyzed with the High Sensitivity RNA ScreenTape assay on the 2200 TapeStation system and this time the Qubit 2.0 Fluorometer was used by two different analysts (right figure). Both quantitation methods, the High Sensitivity RNA ScreenTape assay and the Qubit 2.0 Fluorometer, yielded comparable results for the tested samples.

The data generated confirms that quantitation results for both RNA ScreenTape assays are in good agreement with data generated with spectrophotometric and fluorometric methods.

Technical Overview: 5991-3426EN

RNA Purity

Detection of genomic DNA contamination



NanoDrop		Agilent 2200 TapeStation system				
Total conc. (ng/µL)	RNA purity (%)	Total conc. (ng/µL)	RNA region conc. (ng/µL)	gDNA region conc. (ng/µL)	RNA purity (%)	Purity accuracy (%)
290	69.0	276	175	98	63.4	91.9
275	72.7	321	221	95	68.8	94.7
250	80.0	246	177	64	72.0	89.9
225	88.9	237	198	37	83.5	94.0
210	95.2	194	175	16	90.2	94.7

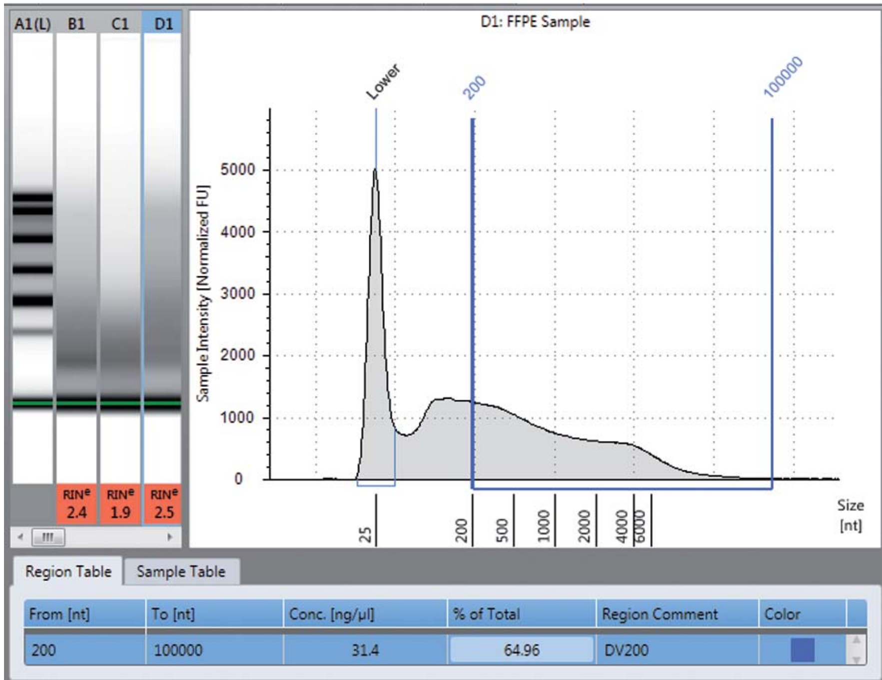
Assay: **RNA ScreenTape Assay**

Abstract: During RNA purification procedures, residual genomic DNA can be present. This can lead to inaccurate RNA quantitation or cause issues with downstream applications. The identification of genomic DNA contamination can therefore be useful in deciding whether further cleanup of the extracted RNA is required. In contrast to some capillary based systems, the 2200 TapeStation system can resolve intact genomic DNA contaminants from the large ribosomal RNA. The figure shows the analysis of rat kidney total RNA spiked with mouse genomic DNA. The genomic DNA contamination results in an additional peak running above the 28S RNA peak. Treatment of the RNA sample with DNase shows that this spiked DNA is removed.

Technical Overview: 5991-3426EN

Analysis of FFPE RNA

DV_{200} Evaluation with RNA ScreenTape Assays



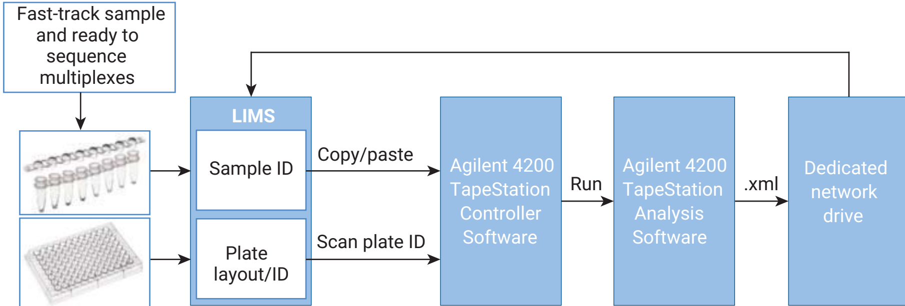
Assay: **RNA and High Sensitivity RNA ScreenTape Assay**

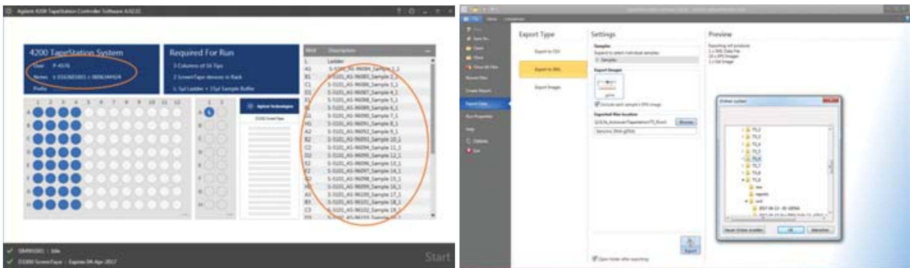
Abstract: The DV_{200} quality metric represents the percentage of RNA fragments above 200 nucleotides and shows a high correlation to the precapture library yield of RNA samples originating from formalin-fixed paraffin-embedded (FFPE) tissue. The RNA and High Sensitivity RNA ScreenTape assays enables fast and easy analysis of FFPE RNA samples, with the TapeStation Analysis 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.

Application note: 5991-8355EN

LIMS Integration

Integration of the 4200 TapeStation system into a laboratory information management system (LIMS)





Abstract: Large core sequencing facilities implement a laboratory information management system (LIMS) to handle all sample relevant data, from pre-analytical information e.g. sample IDs and sample source down to analytical parameters like sample concentration, molarities and sequencing results. The 4200 TapeStation software package allows a seamless LIMS integration. The 4200 TapeStation controller software offers different methods for data import from a database. Sample IDs can be imported from csv-files and additional information like plate ID or lot information of reagents and ScreenTape devices can be entered into the software with an external handheld barcode scanner. Results data can be exported as an .xml file and saved on a dedicated network drive. The .xml-file contains all relevant result data like sizing, concentration, and integrity information for analyzed DNA and RNA samples as well as peak sizes, areas, heights, molarities, and region specific information.

The 4200 TapeStation system offers full automation of RNA and DNA sample quality control and a reduction in hands-on work. In addition, LIMS integration further reduces manual data entry steps resulting in increased efficiency and elimination of a potential source of errors.

Technical Overview: 5991-7984EN

Literature

Application notes and other publications

To download an application note or to find other literature on the 2100 Bioanalyzer and 4200 Tapestation systems visit our websites:
www.agilent.com/genomics/bioanalyzer and
www.agilent.com/genomics/tapestation

Application notes 2100 Bioanalyzer system

DNA analysis

	Publication Number
Next-generation sequencing	
Improving sample quality for SureSelect target enrichment and next-generation sequencing with the High Sensitivity DNA kit	5990-5008EN
Performance characteristics of the High Sensitivity DNA kit for the Agilent 2100 Bioanalyzer	5990-4417EN
DNA quality control of formalin-fixed paraffin-embedded and fresh-frozen tissues prior to target-enrichment and next generation sequencing	5990-0483EN
Low input DNA size selection on the Pippen Prep System using the Agilent 2100 Bioanalyzer system	5990-8382EN
PCR product analysis	
Optimizing real-time quantitative PCR experiments with the Agilent 2100 Bioanalyzer	5989-7730EN
Food analysis	
Use of the Agilent 2100 Bioanalyzer for basmati rice authenticity	5989-6836EN
Strawberry and raspberry fruit differentiation using the Agilent 2100 Bioanalyzer	5990-3327EN
Identification of different meat species by the Agilent Fish ID solution on the Agilent 2100 Bioanalyzer	5990-8452EN

RNA analysis

Analysis of total RNA	
RNA Integrity Number (RIN) - Standardization of RNA quality control	5989-1165EN
Simplified DV_{200} Evaluation with the 2100 Bioanalyzer System	5991-8287EN
Assessing integrity of plant RNA with the Agilent 2100 Bioanalyzer	5990-8850EN
Assessing Integrity of Insect RNA	5991-7903EN

Analysis of Small RNA

Analysis of miRNA content in total RNA preparations using the Agilent 2100 Bioanalyzer	5989-7870EN
Agilent integrated solutions for design, synthesis, and quality control of guide RNA for CRISPR-Cas9 genome editing workflows	5989-8539EN

Genome Editing

Analysis of total RNA

Agilent integrated solutions for design, synthesis, and quality control of guide RNA for CRISPR-Cas9 genome editing workflows	5991-7557EN

Protein analysis

Protein purification

Monitoring protein fate during purification with the Agilent 2100 Bioanalyzer	5990-6153EN

Analysis of modified proteins

Analysis of PEGylated proteins using the Agilent 2100 Bioanalyzer	5990-9593EN

High sensitivity protein analysis

Performance characteristics of the High Sensitivity Protein 250 assay	5989-8940EN
Immunoprecipitation and the High Sensitivity Protein 250 Assay	5990-4097EN
Quantification strategies with the High Sensitivity Protein 250 assay	5989-8941EN

Antibody analysis

Protein analysis with the Agilent 2100 Bioanalyzer— An overview of the protein kit portfolio	5990-5283EN

Food analysis

Rapid wheat varietal identification using the Agilent 2100 Bioanalyzer and automated pattern-matching	5989-7735EN
Milk protein analysis with the Agilent 2100 Bioanalyzer and the Agilent Protein 80 kit	5990-8125EN

Application notes 2200 and 4200 TapeStation systems

DNA analysis	Publication Number
Next-generation sequencing	
Impact of gDNA Integrity on the Outcome of DNA Methylation Studies	5991-6427EN
The DNA Integrity Number (DIN) Provided by the Genomic DNA ScreenTape Assay Allows for Streamlining of NGS on FFPE Tissue Samples	5991-5360EN
Use of the Agilent 4200 TapeStation System for Sample Quality Control in the Whole Exome Sequencing workflow at the German Cancer Research Center (DKFZ)	5991-7615EN
Quality Control for Agilent SureSelect^{QXT} WGS Library Preparation	5991-8191EN
Evaluating the Agilent 4200 TapeStation System for High Throughput Sequencing Quality Control	5991-6892EN
PCR product analysis	
Quality Control of Single Cell DNA Samples with the Agilent D5000 ScreenTape Assays for the Agilent 2200 TapeStation System	5991-5259EN
Genomic DNA	
The DNA Integrity Number (DIN) Provided by the Agilent 2200 TapeStation System is an Ideal Tool to Optimize FFPE Extraction	5991-5246EN
Evaluating the Agilent 4200 TapeStation System for High Throughput Sequencing Quality Control	5991-6892EN
Analysis of high molecular weight genomic DNA using the Agilent 2200 TapeStation and Genomic DNA ScreenTape	5991-1797EN
Quality Control for Agilent SureSelect^{QXT} WGS Library Preparation	5991-8191EN
Integrating the DNA Integrity Number (DIN) to Assess Genomic DNA (gDNA) Quality Control Using the Agilent 2200 TapeStation System	5991-5442EN

RNA analysis

Gene Expression Analysis

A Systematic Approach to Optimize Real-Time Quantitative RT-qPCR Experiments with the Agilent 2200 TapeStation System — 5991-4971EN

Analysis of total RNA

Evaluating the Agilent 4200 TapeStation System for High Throughput Sequencing Quality Control — 5991-6892EN

Performance of the Agilent RNA ScreenTape and the High Sensitivity RNA ScreenTape Assay for the Agilent 2200 TapeStation System — 5991-3426EN

Quality Control for SureSelect Strand-Specific RNA Library Preparation Using the Agilent 2200 TapeStation System — 5991-4116EN

RNA Quantitation

Performance of the Agilent RNA ScreenTape and the High Sensitivity RNA ScreenTape Assay for the Agilent 2200 TapeStation System — 5991-3426EN

RNA Purity

Performance of the Agilent RNA ScreenTape and the High Sensitivity RNA ScreenTape Assay for the Agilent 2200 TapeStation System — 5991-3426EN

Analysis of FFPE RNA

DV_{200} Evaluation with RNA ScreenTape Assays — 5991-8355EN

LIMS integration

LIMS integration of the Agilent 4200 TapeStation System — 5991-7984EN

Applications for DNA, RNA, and Protein Analysis

This compendium highlights key applications using the Agilent 2100 Bioanalyzer and 4200 TapeStation systems.

From next generation sequencing, genomic DNA, RNA, genome editing to protein analyses, this compilation of applications will help you accelerate discovery and support your research.

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