Comparing the Agilent 2100 Bioanalyzer performance to traditional DNA analysis techniques

Application Note

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Abstract

This application note compares the performance of the Agilent 2100 bioanalyzer to the performance of the most commonly used techniques for nucleic acid separation, detection and quantification. A standardized mass ladder and two restriction digests were used in this study for comparisons based on sensitivity, accuracy and reproducibility. Separations were performed on the Agilent 2100 bioanalyzer in conjunction with the DNA 7500 and 12000 LabChip® kits and by electrophoresis on precast 1 % agarose gels stained with ethidium bromide or SYBR®Gold nucleic acid stain. Gels were imaged and analyzed with a fluorescence gel imaging system. In addition, samples were quantitated through fluorescence measurements made with the PicoGreen™ dsDNA quantitation kit. The advantages of better detection sensitivity, sizing accuracy and reproducibility, coupled with a rapid and automated analysis indicate that analyses performed with the Agilent 2100 bioanalyzer are superior to those using the leading alternatives.

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Introduction

As the frontiers of genetic, biological and biochemical research continue to evolve, the modern laboratory must be conscious of the time and cost of analyses, as well as the quality and reproducibility of data. One of the most common yet tedious techniques performed in biological labs is the characterization of DNA via slab gel electrophoresis.

This technique is typically qualitative. For a simple check of the samples a polaroid photograph taken over a UV light source is sufficient to identify general sample quality and to estimate fragment size and quantity against molecular size and mass markers. However, more quantitative measurements, which are frequently necessary for samples used in cloning, sequencing or gene expression experiments, require imaging apparatus. The most popular options are fluorescence scanners or digital cameras, which interface with gel analysis software designed to accurately determine the size and amount of the DNA in specific bands. Typically, this imaging software requires a significant amount of manual intervention to generate results. Although quantitative information on individual sample fragments can be extracted from gels, it is not very reliable and further measurements must be made on a fluorimeter or UV spectrophotometer to ensure accurate concentrations. These instruments, however, only provide information on the concentration of the total sample. They do not provide any information regarding sample constituents or potential contaminants, and use of these instruments can be very time intensive while consuming large quantities of precious sample. Until now, there have not been any alternative technologies available to reduce the time, effort and amount of sample needed to size and quantitate DNA fragments.

The Agilent 2100 bioanalyzer is the first commercially available chip based nucleic acid analysis system. Through the use of microfluidic technology, only 1 µl of nucleic acid sample is required for separation in micro-channels that are filled with a sieving polymer and a fluorescent dye. When an electrical voltage is applied to the microchip, the sample migrates through these micro-channels etched in the chip surface. As sample moves, DNA fragments of different sizes separate according to their mass. Intercalating dye within the sieving matrix allows the migrating DNA fragments to be detected. A PC connected to the instrument controls the separations, records the fragment sizes and concentrations, and allows real-time data analysis and manipulation. This integration streamlines the entire workflow process (table 1).

Analysis with the Agilent 2100 bioanalyzer yields several important advantages compared to traditional separation, imaging and analysis techniques. Analysis of DNA fragments, including chip preparation, separation, detection and data analysis, is performed in a more timely manner. Prepackaged kits, standardized sample preparation and automated analysis yield more accurate and reproducible data due to decreased manual intervention. Three different DNA kits are available to size a broad range of DNA fragments and cover a range of fragment sizes from 25 to 12000 bp. Most importantly, the bioanalyzer has better sensitivity and performance than the common competitive techniques currently available, using minimal amounts of sample.

	Chip	Gel
Preparation (incl. loading of samples)	5 min.	10 min.
Separation	30 min.	30–180 min.
Staining/destaining	-	15 min.
Scanning/analysis	-	20 min.
Total	**35 min.**	**75–225 min.**

Table 1
Comparison of the time requirements to run chips on the Agilent 2100 bioanalyzer versus comparable alternative techniques. For precise quantitation of samples qualitatively checked on agarose gels, solution-based fluorescence measurements are often used. These methods require an additional 45 minutes to prepare the samples, perform the measurements and analyze the data.

Materials and Methods

Chemicals and Reagents

pBR322 digested with Bst NI, and Dra I restriction enzyme were purchased from New England Biolabs (Beverly, MA). Low DNA Mass Ladder and 1 kb Plus DNA Ladder were obtained from Life Technologies (Rockville, MD). Adenovirus 2 DNA and 6X Blue Ficol loading dye were acquired from Sigma (St. Louis, MO). PicoGreen™ dsDNA quantitation kit and 10,000X SYBR® Gold nucleic acid stain were purchased from Molecular Probes Inc. (Eugene, OR). Ethidium bromide at the concentration of 10 mg/ml, 0.5M EDTA and 10X TBE were ordered from Amresco (Solon, OH). Reliant 1% agarose precast gels were obtained from Promega (Madison, WI). DNA 7500 LabChip kits and DNA 12000 LabChip kits were supplied by Agilent Technologies.

Sample Preparation and Quantitation

The Dra I digestion of Adenovirus 2 DNA was prepared by combining 40 µg of Ad2 with 246 units of Dra I for three hours at 37° C. The reaction was stopped by adding EDTA to a final concentration of 10 mM. The Ad2/DraI sample was then aliquoted and diluted with deionized water to the following concentrations: 250 ng/µl, 100 ng/µl, 50 ng/µl, 10 ng/µl, 2.5 ng/µl and 0.5 ng/µl. The 1 µg/µl stock of pBR322/Bst NI digest was diluted to the following concentrations: 500 ng/µl, 250 ng/µl, 50 ng/µl, 5 ng/µl, 0.5 ng/µl, and 0.05 ng/µl. Low DNA mass ladder was alliquoted at the following concentrations: 117.5 ng/µl, 58.75 ng/µl, 14.69 ng/µl, 3.67 ng/µl, 0.92 ng/µl, and 0.23 ng/µl.

For comparison, all samples and dilutions were quantitated using the PicoGreen™ dsDNA quantitation kit from Molecular Probes, in 200 µl volumes on a 96-well micro titer plate. Kit reagents were prepared according to the recommended PicoGreen™ protocols. Fluorescence measurements were made on the CytoFluor™ II Microplate Fluorescence Reader (PerSeptive Biosystems). The concentrations determined by the fluorescence measurements were used as the reference sample concentrations. Quantitation measurements from the other comparative techniques were compared against these values.

Chip Preparation

The Low DNA Mass Ladder and pBr322/Bst NI samples were analyzed with the DNA 7500 LabChip kit for sizing and quantitation. The Ad2/Dra I digest sample was analyzed with the DNA 12000 LabChip kit. All chips were prepared according to the instructions provided with the DNA 7500 and DNA 12000 LabChip kits.

Gel Preparation, Staining and Imaging

Loading dye was diluted 1:5 with 1X TBE buffer. One microliter of ladder or sample was mixed with five microliters of diluted dye, and then loaded onto a 1 % agarose precast gel according to the Reliant gel instructions. The gels were run in 1X TBE at 100 V for approximately 1.5 hours. Following separation, the gels were stained with either ethidium bromide or SYBR®Gold nucleic acid stain for 15 minutes. The staining buffer was made by adding 5 µl of 10 mg/ml ethidium bromide or 10,000X SYBR® Gold to 50 mL of 1X TBE. After staining, the gels were destained to remove background fluorescence by rinsing with 50 ml of 1X TBE, twice. Immediately following destaining, the gels were imaged on the FluorImager 595 (Molecular Dynamics) 610 RG filter. The PMT voltage was set to 900V for a two minute scan at normal detection sensitivity. Gel images were generated with a digital resolution of 16 bits per pixel, with the pixel size set to 100 microns. The gel image was then analyzed with the FragmeNT software.

Results and Discussion

In many biological experiments where nucleic acids are manipulated in multi-step procedures, the introduction of impurities, non-specific generation of PCR products, and incomplete digests, can have drastic effects on the outcome of an experiment. The ability to detect and accurately characterize small amounts of fragments enables the scientist to optimize reactions and actively guide the course of experimentation. The DNA 7500 LabChip kit is designed to size and quantitate double-stranded DNA fragments from 100–7500 bp while the DNA 12000 LabChip kit sizes fragments as large as 12 kbp.

Sensitivity and Linearity

System performance was evaluated by comparing the results obtained with the Agilent 2100 bioanalyzer to gel electrophoresis on 1 % agarose gels stained with either SYBR® Gold or ethidium bromide. Comparison between the chip and the gel images of pBR322/Bst NI digest dilutions run at concentrations ranging from 500 ng/µl to 0.05 ng/µl (figure 1) clearly shows that the bioanalyzer is able to detect fragments that cannot be positively identified on the agarose gels. Dilutions of Low DNA Mass Ladder, which contain standardized fragment concentrations, were used to determine the limits of detection for each system. The bioanalyzer was able to consistently detect 0.02 ng of fragment.

This is five times more sensitive than gels stained with SYBR® Gold and twenty five times more sensitive than gels stained with ethidium bromide, which have detection limits of 0.1 ng and 0.5 ng respectively. Although high sensitivity is desirable, accurate quantitation can only be obtained within the linear range of the assay for the Agilent 2100 Bioanalyzer. Low DNA Mass Ladder fragments ranging from 0.02 ng to 48 ng were used to determine the linearity of the bioanalyzer (figure 2). The DNA 7500 assay was found to be linear from 0.5—50 ng/µl (r^2 =0.9988).

Such high accuracy can be achieved with the Agilent 2100 bioanalyzer because both external standards, in the form of a DNA ladder, and internal standards, in

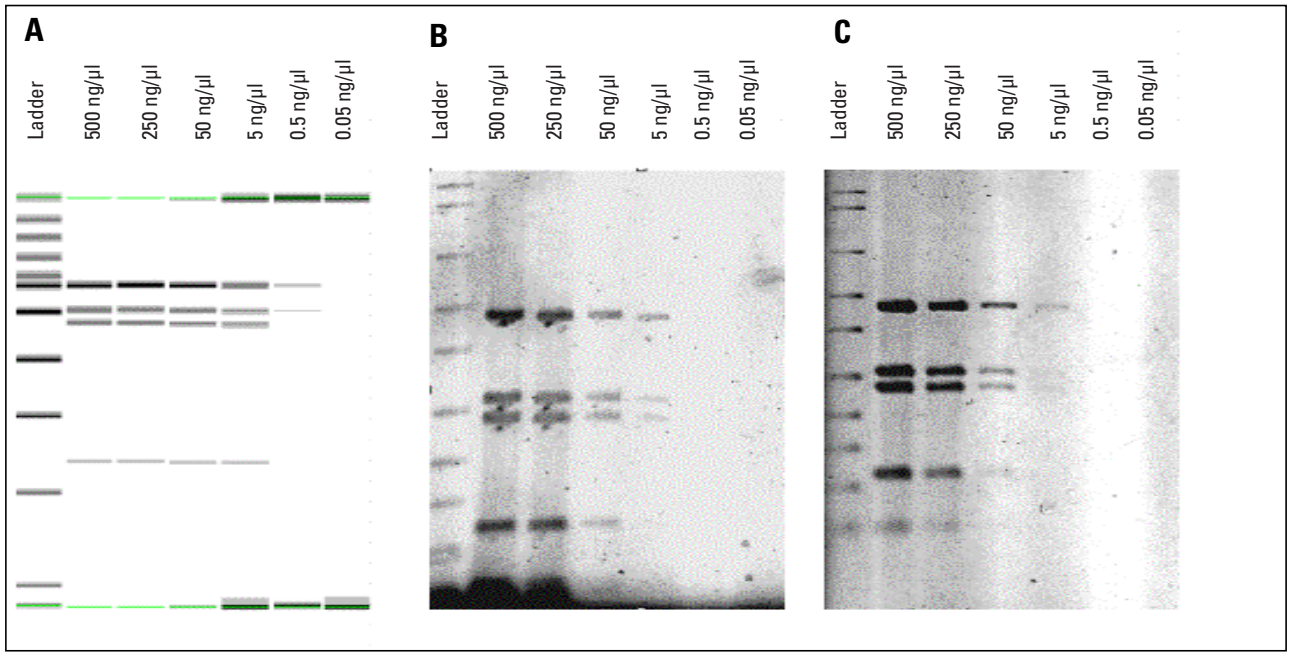

Figure 1
Gel image comparison of pBR332/Bst NI digest dilutions run on: A) the Agilent 2100 bioanalyzer, B) a 1% agarose gel stained with SybrGold™ nucleic acid stain, and C) a 1% agarose gel stained with ethidium bromide. For sizing comparison on the gels, the six pBR322/Bst NI samples were run with the DNA 7500 LabChip kit ladder in lane number 1. Both sets of agarose gels were scanned with the FluorImager™ 595.

the form of mass and size markers which are run in every lane, are used to calibrate the sizing and quantitation of each sample. This eliminates slight differences as each sample is sequentially run through the separation channel. Typically, internal standards are not run with samples on a gel. Additional variability can also occur when loading samples into submarine gels and in the staining following the separation. Subsequently, it is commonly accepted that quantitation off of gels is not a reliable method of accurately measuring sample concentration.

Quantitative Analysis

Accurate and reproducible quantitation is important for many procedures including depositing precise amounts of PCR products onto microarrays, determining the correct amount of cloning insert needed relative to vector for a ligation reaction or calculating the correct amount of DNA template needed for a sequencing reaction. The ability of the bioanalyzer to accurately determine fragment concentration, while simultaneously sizing and checking sample integrity, is a valuable advantage over current competitive technologies.

Quantitation accuracy and reproducibility were determined by analysis of low DNA mass ladder fragments at six concentrations ranging from 1.2 ng/µl to 24.2 ng/µl (table 2). For these measurements the precision was between 85 and 90 %, whereas the percent standard deviation was between 4 and 11 %. The quantitation derived from the agarose gels was considerably less accurate and reproducible (data not shown). Only at concentrations above 50 ng/µl did the accuracy approach 50 %, while reproducibility even at this concentration was very poor due to the high variability in staining.

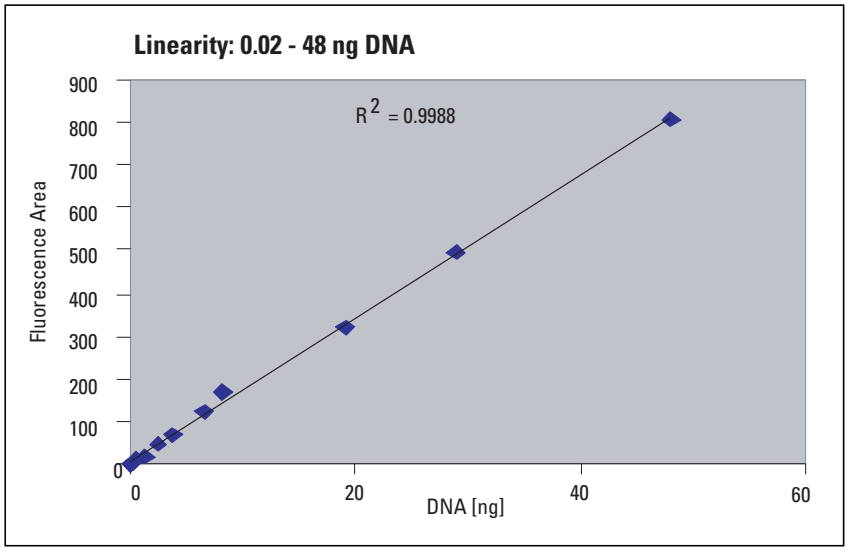

Figure 2
Linear relationship between the expected DNA fragment concentration and the area under the fluorescence electropherogram. Although the assay is linear over three orders of magnitude, the recommended DNA assay specifications for concentration lay between 0.5 ng–50 ng/µl.

Conc. (ng/µl)	Chip conc. (n=60)	% Error	Std. Dev.	% CV
1.2	1.1	9.4	0.13	10.6
2.4	2.1	11.8	0.14	5.9
4.9	4.2	13.7	0.23	4.8
9.3	8.5	13.3	0.43	4.5
14.8	12.8	15.5	0.65	4.4
24.2	21.2	14.4	1.36	5.6

Table 2
Six low mass ladder fragments were analyzed with the Agilent 2100 bioanalyzer to determine the quantitation accuracy and reproducibility of the DNA 7500 assay.

Sizing Analysis

Dilutions of pBR322/Bst NI digest samples, which contain five fragments ranging from 121–1857 bp, were used to determine the sizing accuracy and reproducibility of the DNA 7500 assay compared to the analysis of agarose gels (table 3). On average, the data generated with the bioanalyzer showed lower percentage errors in comparison to agarose gel analysis. The reproducibility of the bioanalyzer was also slightly better than that of the ethidium bromide and the SYBR®Gold stained gels.

The performance of the DNA 12000 LabChip kit for sizing analysis was compared to agarose gel analysis using dilutions of an Ad2/DraI digest that is comprised of twelve fragments ranging from 119 bps to 9228 bps. (figure 3.). Not only are the bands in the bioanalyzer gel-like image more defined and more uniform, all of the bands are also consistently detected. (figure 4). The 119 bp fragment does not resolve from the 149 bp fragment on the agarose gels, even when 250 ng of sample are loaded onto the gel. The ability to detect and accurately size both very small and very large digest fragments with the same assay is a unique advantage of the bioanalyzer. Table 4 contains the data comparing the DNA 12000 assay performance to the gel analysis with FragmeNT. The sizing accuracy of all three techniques was very good. The DNA 12000 assay showed similar reproducibility compared to agarose gels stained with SYBR®Gold and ethidium bromide.

	Chips DNA 7500 assay				1 % agarose gels SYBR®Gold stained				1 % agarose gels EtBr stained			
Size (bp)	Chip Size	% Error	Std. Dev.	% CV	Gel Size	% Error	Std. Dev.	% CV	Gel size	% Error	Std. Dev.	% CV
121	112	7.2	5.9	5.2	133	-9.7	12.9	9.7	115	5.2	4.6	4.0
383	380	0.9	1.3	0.3	459	-19.8	71.2	15.5	399	-4.3	17.4	4.4
929	932	-0.4	3.0	0.3	996	-7.2	19.9	2.0	958	-3.1	29.0	3.0
1058	1073	-1.4	53.0	4.9	1128	-6.6	28.1	2.4	1097	-3.7	35.6	3.2
1857	1650	11.2	45.3	2.8	1985	-6.9	34.3	1.7	1921	-3.4	46.1	2.4

Table 3
Five fragments from the pBR322/ Bst N I digest were used to compare the sizing accuracy and reproducibility of the Agilent 2100 bioanalyzer to that achieved by gel analysis.

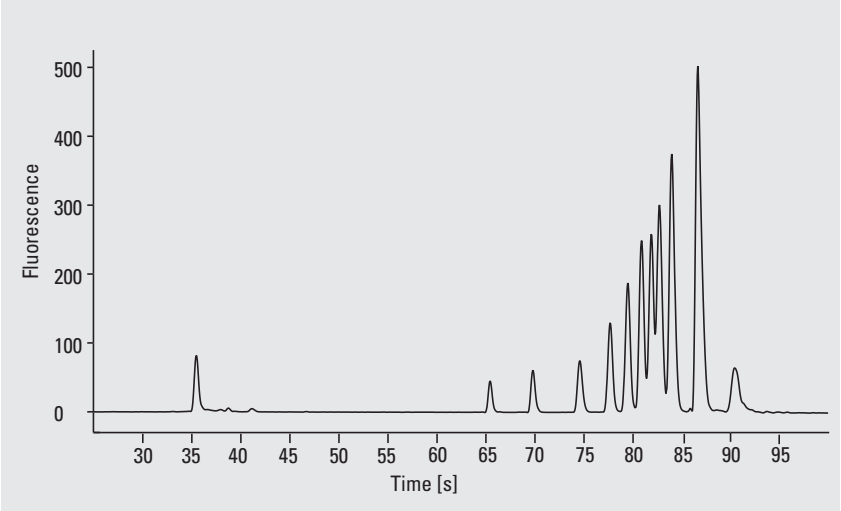

Figure 3
Electropherogram of Ad2/DraI at 50 ng/µl analyzed on the Agilent 2100 bioanalyzer.

Conclusion

The Agilent 2100 bioanalyzer shows excellent performance for quantitative analysis and sizing of DNA fragments. The advantages of the bioanalyzer boldly stand out with respect to sensitivity, linear dynamic range and sizing capability over a broad range of fragment sizes. The bioanalyzer performed fragment quantitation at 85 % or better, with a percent standard deviation less than or equal to 10 %. The same precision could not be attained with the fluorescence scanner. Over a size range of 100 to 12000 bps, the percent standard deviation of both the DNA 7500 and DNA 12000 assays

was found to be 5 % or lower, while the accuracy was 90 % or better. Scanned gels stained with ethidium bromide or SYBR® Gold had typically higher percentage sizing errors. The performance of the bioanalyzer was found to be superior to agarose gel analysis. Additionally, the bioanalyzer consumes considerably less sample and is less labor intensive. As the separations are performed, the DNA fragments are detected and analyzed in real-time, and the digital data storage facilitates easy data exchange. There are no additional staining, de-staining or imaging steps before data extraction. Automation of both the separation and data analysis makes the Agilent 2100 bioanalyzer easy to use, while the superior performance makes it an ideal tool for the analysis of nucleic acids.

	Chips DNA 12000 assay				1 % agarose gels SYBR®Gold stained				1 % agarose gels EtBr stained			
Size (bp)	Chip (n=216)	% Error	Std. Dev.	% CV	Gels (n=54)	% Error	Std. Dev.	% CV	Gels (n=54)	% Error	Std. Dev.	% CV
119	113	5.0	0.5	0.4								
149	153	-2.5	0.6	0.4	137	8.1	2.3	1.7	1.3	12.3	2.9	2.2
641	656	-2.2	12.9	2.0	672	-4.9	6.9	1.0	671	-4.7	7.6	1.1
815	831	-1.9	15.3	1.8	829	-1.7	6.1	0.7	819	-0.5	21.9	2.7
1195	1205	-0.1	37.4	3.1	1261	-5.5	32.5	2.6	1232	-3.1	28.4	2.3
2058	1937	5.9	99.9	5.2	2120	-3.0	23.4	1.1	2099	-2.0	15.2	0.7
2800	2626	6.2	58.1	2.2	2905	-3.8	118.7	4.1	2848	-1.7	27.4	1.0
3588	3343	6.8	36.4	1.1	3683	-2.7	55.7	1.5	3634	-1.3	59.8	1.7
4182	4145	0.9	32.8	0.8	4316	-3.2	49.6	1.2	4247	-1.6	56.3	1.3
4845	4719	2.6	31.3	0.7	4805	0.8	178.1	3.7	4732	2.3	319.5	6.8
6297	5793	8.0	78.9	1.4	6642	-5.8	172.2	2.6	6332	-0.6	366.6	5.8
9228	8508	7.8	338.7	4.0	10367	-12.4	206.2	2.0	10349	-12.1	262.0	2.5

Table 4
System performance of the Agilent 2100 bioanalyzer versus that of scanned agarose gels with respect to accuracy and reproducibility, over a broad range of Ad2/Dra I digest fragment sizes. Note that the 119 bp fragment was only detected by the Agilent 2100 bioanalyzer.

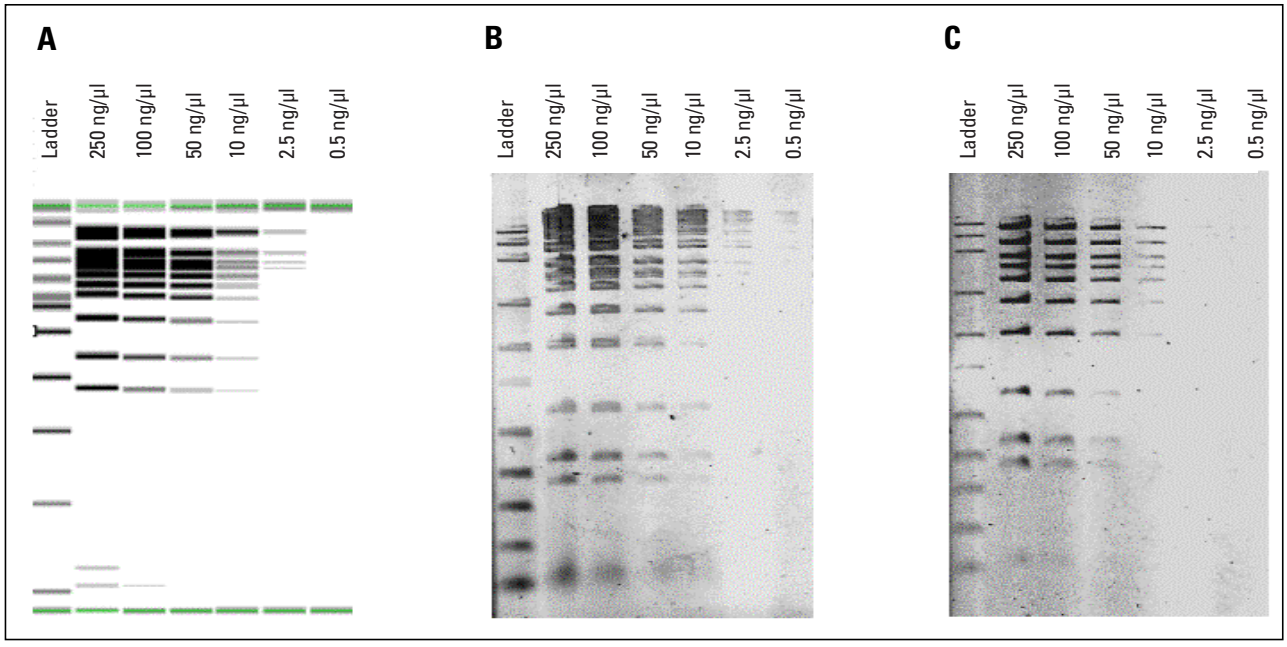

Figure 4
Gel image comparison of adenovirus 2 DraI digest dilutions run on A) the Agilent 2100 bioanalyzer, B) a 1% agarose gel stained with SYBR®Gold nucleic acid stain, and C) a 1% agarose gel stained with ethidium bromide. For sizing comparison on the gels, the six Ad2/Dra I samples were run with the DNA 12000 LabChip kit ladder in lane number 1. Both sets of agarose gels were scanned with the FluorImager™ 595.

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