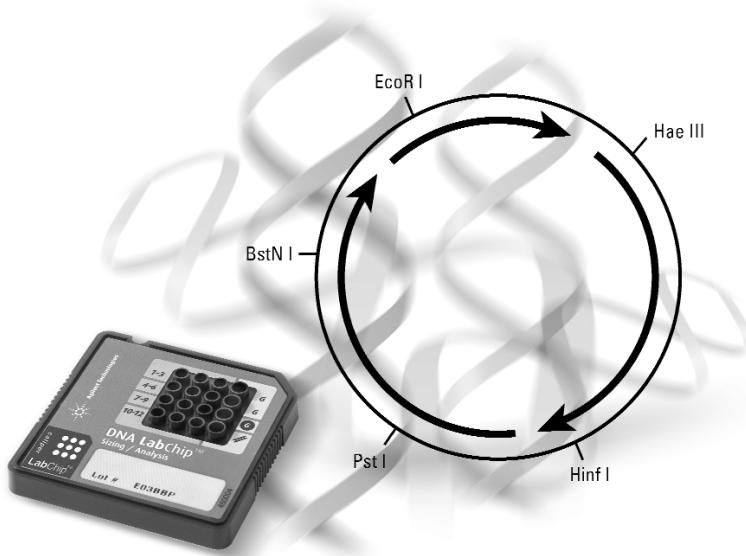


High precision restriction fragment sizing with the Agilent 2100 Bioanalyzer

Application Note

Odilo Mueller



Abstract

This application note describes how the DNA 12000 LabChip® kit can be used with the Agilent Technologies 2100 bioanalyzer to size DNA fragments generated by restriction enzyme digestion. Sizing accuracy was found to be greater than 92 % for all samples analyzed. Comparison of the chip-based separations to those obtained using agarose gel electrophoresis indicates that the DNA 12000 assay accurately sizes fragments over a wider range than can be usually analyzed on a slab gel.



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Introduction

At present, slab gel electrophoresis (SGE) is the most widely used technique for restriction fragment analysis. While SGE is a relatively inexpensive and easy-to-use technique, the amount of accurate sizing information that can be derived without additional effort from slab gels is limited. Typically, the fragment length is estimated by the scientist through visual comparison to appropriate sizing ladders which are run in separate lanes on the gel. Gel-scanning systems are available for these applications. They are used to scan gels after staining with an appropriate dye. However, these systems are expensive, require manual intervention, and the use of several individual hardware components.

The limitations of slab gel electrophoresis can be overcome with the Agilent 2100 bioanalyzer which is the first commercially available chip-based nucleic acid separation system. This system performs capillary electrophoresis on micro-fabricated channels and carries out detection as well as online data evaluation in an automated manner. The Agilent 2100 bioanalyzer is connected to a PC for run control and automated data analysis. Several kits are available to analyze a variety of nucleic acid sample types. The DNA 12000 LabChip Kit is best suited for the size determination of restriction digests with fragment sizes in the range of 100 to 12000 base pairs. Since restriction

digests yield fragments with the same molarity, quantitative analysis is usually not needed*.

Analysis of restriction fragment digests on the Agilent 2100 bioanalyzer provides several important advantages compared to traditional slab gel electrophoresis. Since a short separation channel is employed and a high electrical field strength is applied, the speed of analysis is dramatically increased compared to slab gel electrophoresis. The speed of analysis results in an increased sample throughput. The instrument is equipped with a fluorescence detection system resulting in superior detection sensitivity. The prepackaged reagents and kits are used in conjunction with standardized protocols and result in more reproducible data. These kits also help to improve the overall reproducibility between different runs, chips, and instruments. Compared to data assessment with gel scanning systems, the amount of manual work is significantly reduced and even data analysis is performed in an automated manner. Sample and reagent consumption in the range of one to a few microliters minimizes exposure to hazardous materials and reduces the amount of waste material.

* For quantitative analysis of dsDNA, use the DNA 7500 kit described in publication number 5968-7496E

Materials and methods

Agilent 2100 bioanalyzer instrument and software

All chip-based separations were performed on the Agilent 2100 bioanalyzer, which was controlled by dedicated software running on a PC. The Agilent 2100 bioanalyzer software package includes data collection, presentation and interpretation functions. Data can be displayed as a gel-like image and/or as electropherogram(s) as shown in figure 1. Additionally, sizing data is presented in tabular

form and can be easily exported to various spreadsheet programs. A number of software tools are available for data manipulation and comparison.

The Agilent 2100 bioanalyzer contains high voltage power supplies, each of which is connected to a platinum electrode. These electrodes allow the instrument to perform multiple injections and other fluid manipulations from specific sample wells. The instrument uses fluorescence detection, monitoring fluorescence between 670 nm and 700 nm.

Chip Preparation

All chips were prepared according to the instructions provided with the DNA 12000 LabChip kit. Each kit includes 25 chips and the following reagents: gel matrix, dye concentrate, DNA markers, DNA sizing ladder, syringe, and spin filters. The gel-dye mix was prepared by mixing 400 μ l of the gel matrix with 20 μ l of the dye concentrate and the mixture was filtered through a spin filter. The separation chip was filled with the gel matrix/dye mixture and 5 μ l of the markers were added to each sample well. After adding 12 samples (1 μ l each) to the sample wells and the DNA sizing ladder (1 μ l) to the assigned ladder well, the chip was vortexed and run on the Agilent 2100 bioanalyzer.

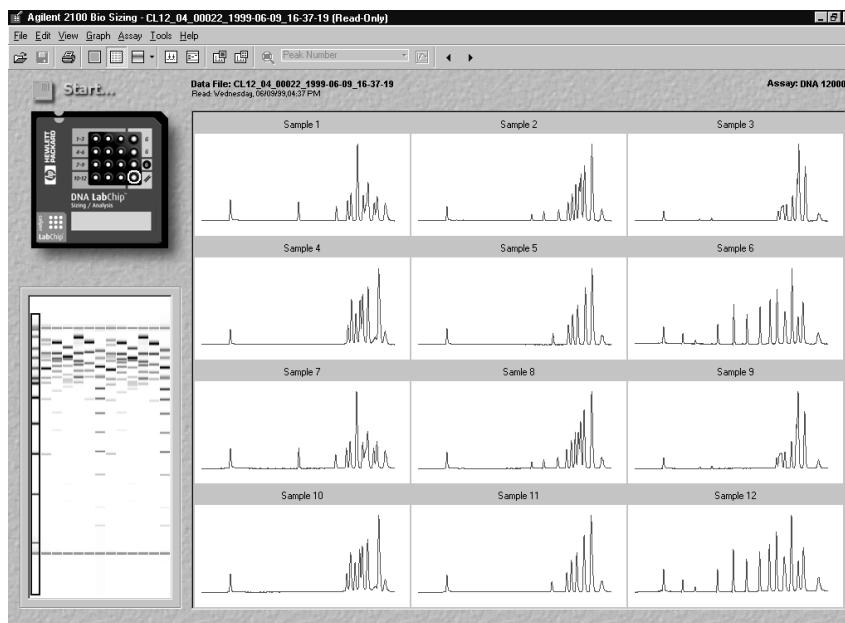


Figure 1

The DNA 12000 assay as displayed in the Agilent 2100 biosizing software. Data is presented both as electropherograms and a gel-like image. The screen shows several commercially available sizing standards as well as in-house prepared restriction digests.

Chemicals and reagents

Adenovirus 2 DNA was purchased from Sigma-Aldrich Corp. (St. Louis, MO). The restriction enzymes as well as the 1 kbp ladder were obtained from New England Biolabs Inc. (Beverly, MA). Adenovirus 2 DNA was digested with Dra I or Bgl II using standard conditions, at a final DNA concentration of 50 ng/ μ l. Following digestion, restriction enzymes were inactivated by the addition of EDTA to a final concentration of 10 mM. Aliquots of 1 μ l were analyzed on the Agilent 2100 bioanalyzer using the DNA 12000 LabChip kit from Agilent Technologies GmbH (Waldbronn, Germany).

Results and discussion

The Agilent 2100 bioanalyzer analyzes 12 DNA samples in less than 30 minutes in a sequential manner and the results for each sample can be viewed after completion of the run. The DNA 12000 assay can be used to size double stranded DNA fragments ranging in size from 100 to 12000 base pairs with a sizing accuracy better than 85 %. Run to run and chip to chip reproducibility is ensured by means of external standards (DNA sizing ladder) and internal standards (DNA markers).

The assay is compatible with commonly used restriction digest buffers so that no desalting or other sample pre-treatment is necessary. Some restriction digests may require dilution of the sample if the total fragment concentration significantly exceeds 50 ng/ μ l. While agarose gels often show good performance in certain size ranges, the DNA 12000 assay allows accurate sizing over a wide range of fragment lengths.

Chip versus gel analysis of 1 kbp ladder and restriction enzyme digests of adenoviral DNA

Sizing accuracy was checked by running a DNA size standard and different restriction fragment digests on a 1 % agarose gel as well as on the Agilent 2100 bioanalyzer. The samples contained various fragments ranging from 119 to 10000 base pairs in length. The individual fragments of each sample are listed in table 1.

For the gel analysis, a 1 % agarose gel stained with ethidium bromide was used and the results are displayed in figure 2. In general, the fragments of all samples were separated, with only four comigrating fragments (fragments 5088 and 5228 and fragments 1547 and 1549 in Adenovirus 2/Bgl II). However, in order to separate the longer DNA fragments, the runtime had to be extended which resulted in the shorter DNA fragments running off the gel and not being detected (see highlighted lines in table 1). In the present example fragments shorter than 500 bp were not detected.

1 kbp ladder	Adenovirus 2/Dra I	Adenovirus 2/Bgl II
10000 bp	9228 bp	7684 bp
8000 bp	6297 bp	5582 bp
6000 bp	4845 bp	5228 bp
5000 bp	4182 bp	5088 bp
4000 bp	3588 bp	3322 bp
3000 bp	2800 bp	2284 bp
2000 bp	2058 bp	1757 bp
1500 bp	1195 bp	1549 bp
1000 bp	815 bp	1547 bp
500 bp	641 bp	1270 bp
	149 bp	351 bp
	119 bp	275 bp

Table 1
Fragment sizes of samples used to test the performance of the DNA 12000 assay

The same three samples were also analyzed using the DNA 12000 assay. No sample pre-treatment was necessary prior to loading the digested samples onto the chip. (figures 3 A, B, and C). While the chip-based separation showed similar resolution to the slab gel method (only one additional fragment was not separated), even the short fragments were detected in the sample. This improvement versus the slab gel method stems from the fact that all fragments pass by the detection window. The dynamic detection range of the detection system in the Agilent 2100 bioanalyzer is sufficient to detect even minute amounts of sample (LOD \approx 0.05 ng/ μ l). For the three samples,

the sizing accuracy was greater than 92 %. Typically, sizing accuracy is above 90 %. In the rare case where DNA fragments exhibit strong sequence dependent migration, sizing accuracy remains greater than 85 %.

The wide range of DNA concentrations and DNA fragment sizes that can be analyzed on a single chip makes the DNA 12000 assay a versatile and easy-to-use tool for the analysis of restriction fragment digests. Additionally, the automated on-line size analysis and tabular output for each sample is convenient and saves time in comparison to conventional gel technology.

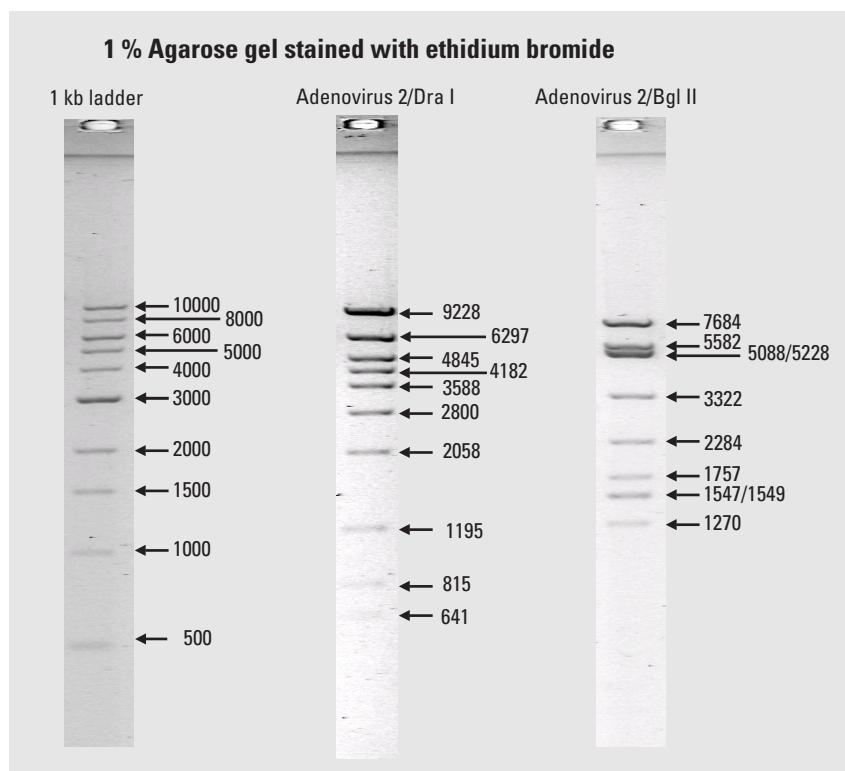


Figure 2
Analysis of three different samples on a 1 % agarose gel containing ethidium bromide. Fragments below 500 bp were not detected.

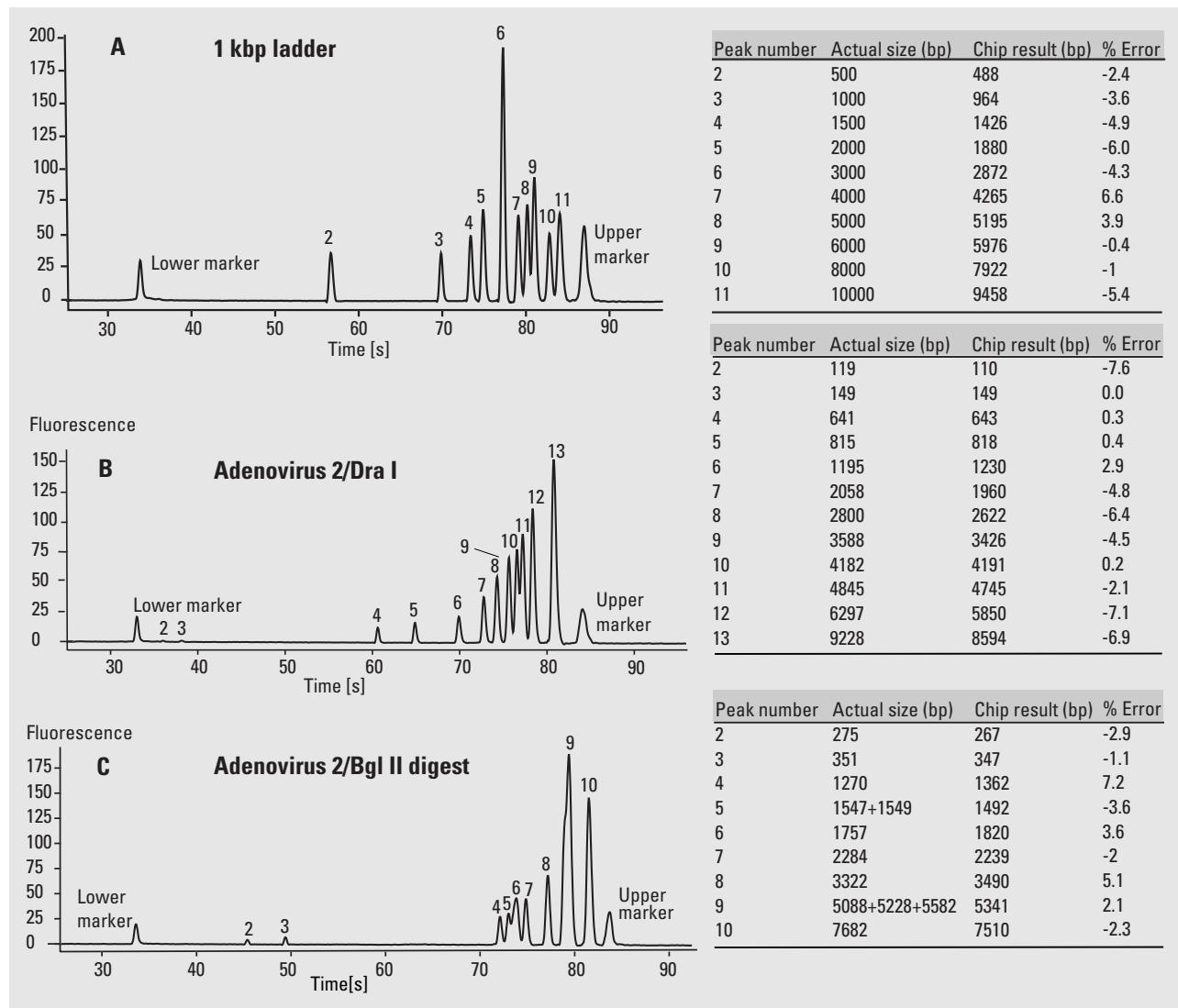


Figure 3

Sizing results of the 3 samples from figure 3 as obtained from the Agilent 2100 bioanalyzer: A) 1 kbp ladder, B) Adenovirus 2/Dra I, C) Adenovirus 2/Bgl II. All fragment sizes are detected with a sizing accuracy > 92 %.

Conclusion

The Agilent 2100 bioanalyzer shows excellent performance for sizing of restriction fragments. The use of internal and external DNA markers allows analysis of multiple samples through a single separation channel with very high reproducibility and reliability. Data precision is comparable or superior to slab gel analysis, whereas analysis times are greatly reduced using the Agilent 2100 bioanalyzer. Automation of both separation and data analysis makes the Agilent 2100 bioanalyzer versatile and easy to use. In addition to the analysis of restriction fragments with the DNA 12000 LabChip kit, the instrument platform can be used for other nucleic acid analyses. Initial kits are available for sizing and quantitation of PCR fragments (DNA 7500 LabChip kit) and for quantitation and integrity/purity check of total RNA and mRNA samples (RNA 6000 LabChip kit).

Odilo Mueller is an application chemist based at Agilent Technologies, Waldbronn, Germany.

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<http://www.agilent.com/chem/labonachip>

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