Sizing of Fragments with the WAVE™ Nucleic Acid Fragment Analysis System

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Introduction

Liquid chromatography (LC) is a powerful technique for separation and quantitation of nucleic acids due to its high-resolving capability, short analysis time, and ease of recovery of DNA fragments for subsequent studies. The WAVE™ System combines the precision of ion-pair reversed-phase LC with automated sampling, fragment collection, data acquisition and reporting functions. Fragments are collected and subjected to further molecular analysis such as subcloning, amplification and sequencing (see ADS Biotec Application Note No. 104).

DNA fragment sizing is required for quality control (QC) of sequencing templates; quantitation and sizing of DNA fragments; "yes/no" assays for the presence of a DNA fragment in genotyping (see ADS Biotec Application Note No. 106); optimization of PCR* conditions; and QC on PCR* products. Sizing of DNA fragments by ion-pair reversed-phase LC is independent of fragment sequence and sequence-related secondary structures of the molecule.

Methods for DNA Fragment Sizing

The method in Table 1 has been developed for analysis of double stranded DNA fragments with maximum length of 600 bp. Figure 1

Time (min)	Buffer A: 0.1 M TEAA (%)	Buffer B: 0.1 M TEAA, 25% ACN (%)	Flow Rate (mL/min)
0	65	35	0.75
3	45	55	
10	35	65	
13	35	65	
14	0	100	
15.5	0	100	
16.5	65	35	

Table 1. Protocol for DNA fragment sizing based on pUC18 HaeIII *digest analysis with the WAVE™ System (DNASep™ column temperature: 50°C).*

Time (min)	Buffer A: 0.1 M TEAA (%)	Buffer B: 0.1 M TEAA, 25% ACN (%)	Flow Rate (mL/min)
0	65	35	0.75
3	45	55	
10	35	65	
10.1	0	100	
11	0	100	
11.1	65	35	
15	65	35	

Table 2. Optimized protocol for DNA fragment sizing of PCR fragments up to 400 bp with the WAVE™ System (DNASep™ column temperature: 50°C).*

Fragment size, bp	% Buffer B
0	19.2
20	30.1
40	37.3
60	42.5
80	46.3
100	49.3
120	51.6
140	53.6
160	55.2
180	56.6
200	57.7
220	58.7
240	59.6
260	60.4
280	61.1
300	61.7
320	62.3
340	62.8
360	63.2
380	63.7
400	64.0

Table 3. Guidelines for method development in sizing of DNA fragments.

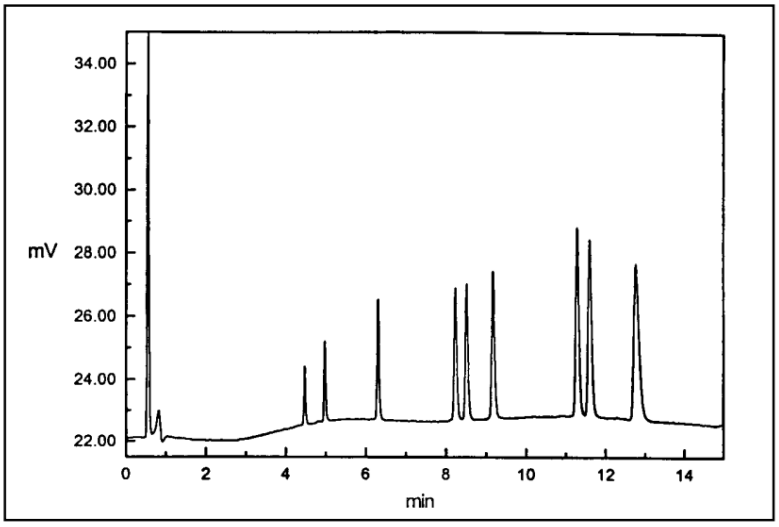

Figure 1. Double-stranded DNA fragment sizing of a pUC18 plasmid cut with HaeIII *was carried out using the method in Table 1. Fragments with sizes 80, 102, 174, 257, 267, 298, 434, 458, and 587 bp are shown.*

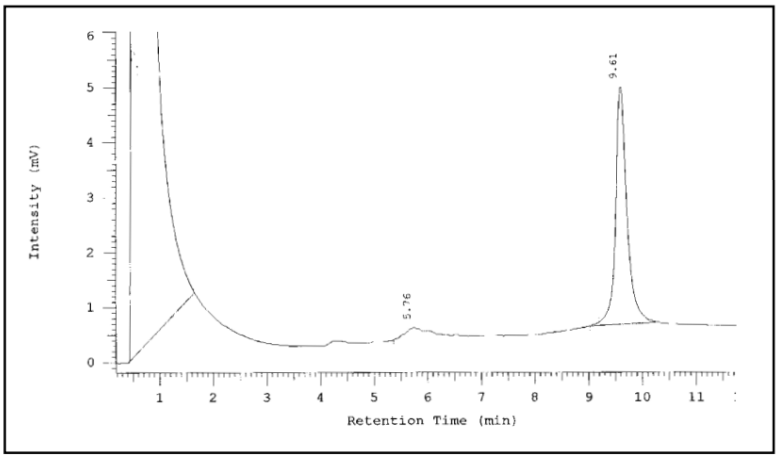

Figure 2. Analysis of a 398 bp PCR fragment carried out using the method listed in Table 2.*

shows fragment sizing of pUC18 plasmid cut with *Hae*III. This method has been optimized (shown in Table 2) for analysis of PCR* fragments up to 400 bp, as illustrated in Figure 2.

Guidelines for DNA Sizing Optimization

Table 3 shows recommended guidelines for method development in DNA fragment sizing with the WAVE™ System.

Peak Identification

The WAVE™ System reports the detection of a DNA fragment by a peak, which is identified by its retention time (RT). The retention time of a peak varies slightly, depending on the conditions under which the instrument is operated. Therefore, it is necessary to use a time window for identification of the peak, i.e., a time interval during which a peak is expected to be registered.

Two types of time windows are used with the WAVE™ System:

1. Percentage (%) Time window: The time interval for a DNA fragment peak is expressed as a percentage of its retention time defined in the Component Table. For example, an 80-bp fragment has a RT of 4.91 min. If 10% is entered in the "Window %" of the Component Table, then the lower limit is:

Fragment, bp	Mean RT, min	STD DEV	STD ERR
80	4.91	0.08	0.03
102	5.44	0.06	0.02
174	6.67	0.06	0.02
257	8.05	0.07	0.03
267	8.27	0.07	0.03
298	8.84	0.06	0.02
434	10.75	0.03	0.01
458	11.03	0.03	0.01

Table 4. Retention time means, standard deviations (STD DEV), and standard errors of the mean (STD ERR) of double-stranded fragments from a pUC18 HaeIII *digest. Method from Table 1 was used. Analysis was performed with JMP software (SAS, Inc., NC), n=7.*

4.91 - (4.91 x 10/100) = 4.42

and the upper limit is:

4.91 + (4.91 x 10/100) = 5.40

Therefore, the window for the 80 bp fragment is from 4.42 min to 5.40 min.

2. Absolute Time window: The time interval for a DNA fragment peak is expressed in minutes. If 0.1 is used in the "Window %" of the component table, then the window for the 80-bp fragment will be from 4.81 min to 5.01 min.

Peak Reproducibility

The precision of ion-pair reversed-phase LC in sizing of DNA fragments and high reproducibility of peak retention times has been thoroughly studied.[1] The standard errors of the mean and standard deviations from mean retention times of DNA fragments with size 80 - 458 bp analyzed on the WAVE™ System are shown in Table 4. It is clear that retention times are highly reproducible in consecutive runs. For high accuracy in DNA fragment sizing experiments, an injection of a molecular weight ladder is recommended after each 20th sample.

References

1. Huber, C.G., Oefner, P.J. and Bonn, G.K. (1995). Rapid and accurate sizing of DNA fragments by ion-pair chromatography on alkylated non-porous poly (styrene-divinylbenzene) particles. *Anal. Chem.* **67**: 578-585.

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