CONF-9506258--1

LASER DESORPTION MASS SPECTROMETRY FOR FAST DNA ANALYSIS

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I. INTRODUCTION

During the past few years, major effort has been directed toward developing mass spectrometry to measure biopolymers because of the great potential benefit to biomedical research. Hillenkamp and his co-workers[1] were the first to report that large polypeptide molecules can be ionized and detected without significant fragmentation when a greater number of nicotinic acid molecules are used as a matrix.[2] This method is now well known as matrix-assisted laser desorption/ionization (MALDI). Since then, various groups have reported measurements of very large proteins by MALDI. Reliable protein analysis by MALDI is more or less well established. However, the application of MALDI to nucleic acids analysis has been found to be much more difficult.

Most research on the measurement of nucleic acid by MALDI were stimulated by the Human Genome Project.[3] Up to now, the only method for reliable routine analysis of nucleic acid is gel electrophoresis. Different sizes of nucleic acids can be separated in gel medium when a high electric field is applied to the gel. However, the time needed to separate different sizes of DNA segments usually takes from several minutes to several hours. If MALDI can be successfully used for nucleic acids analysis, the analysis time can be reduced to less than 1 millisecond. In addition, no tagging with radioactive materials or chemical dyes is needed. In this work, we will review recent progress related to MALDI for DNA analysis.

II. EXPERIMENTAL

Various types of mass spectrometers have been used for MALDI, including Fourier transform,[4] magnetic sector,[5] ion trap,[6] and time-of-flight. The majority of work on MALDI of biomolecules has progressed by using the time-of-flight mass spectrometer (TOFMS), since it has the advantages of simplicity, low cost, and broad coverage of mass range. Up to now, three different types of TOFMS have been used for MALDI of biomolecules: linear time-of-flight, reflectron time-of-flight, and ion trap time-of-flight.[7] A typical linear TOFMS is shown schematically in Fig. 1. A laser beam is used to achieve desorption with or without ionization. Typically a N_2 laser or a Nd-YAG laser is used for ultraviolet (UV) absorption induced desorption.[8] After desorption/ionization, ions are extracted by a one- or two-stage electric field and guided into a field-free drift tube. Ions merging from the drift tube region are subsequently detected by an ion detector, which typically is a microchannel plate, a channeltron, or a discrete dynode multiplier. Some detection schemes used a separate ion impact from which the emitted secondary electrons were subsequently detected by an electron amplification

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device. For the MALDI process, there are usually many more organic matrix ions produced than biomolecular ions of interest. A pulsed ion deflector is often used to deflect matrix ions to prevent the possible saturation of detectors.

III. RESULTS

Recently, we[9] discovered that picolinic acid and aminopicolinic acid are "good" matrices for oligonucleotides. They used picolinic acid to detect ss-DNA and double-stranded (ds) DNA of 190 mer and 190 base pairs. Experimental results are shown in Fig. 2. With aminopicolinic acid as a matrix,[10] ds-DNA of 246 base pairs in size were successfully detected with MALDI (see Fig. 3). When mixtures of 3-hydroxypicolinic acid (3-HPA) and picolinic acid were used for a matrix, ds-DNA of 500 base pairs in size was also successfully detected.[16] Experimental results are shown in Fig. 4. When ds-DNA were in the sample, only ss-DNA ions were observed. This indicates that several hundred hydrogen bonds are broken during the laser ablation of ds-DNA.

VI. APPLICATIONS

Fast DNA analysis by MALDI would have tremendous potential for medical and other applications. DNA sequencing, disease diagnosis, and DNA fingerprinting are among the most important applications.

(A) DNA Sequencing

Due to the very important role of DNA for biological functions, the Human Genome Project has been initiated and extensively pursued to complete the mapping and sequencing of the whole human genome.[3] At the present time, gel electrophoresis is still the only available method for routine DNA sequencing. DNA ladder products need to be produced either by the Sanger approach[12] or the Maxim-Gilbert approach[13] for gel electrophoresis for sequencing. These methods of sequencing usually involve labeling fragments of DNA for identification following gel electrophoresis. Either radioactive labeling such as ^{32}P or ^{35}S or chemical labels such as fluorescent dyes are currently used.[14]

At the present time, routine DNA sequencing in most laboratories relies on gel electrophoresis to separate DNA fragments labeled with radioactive isotopes. The fragments are generated either chemically or enzymatically to represent all possible positions of each of the four nucleotides (A, G, C, and T). Following electrophoresis, the radioactive labels are located by autoradiography. This method is extremely time consuming. With radioactive labeling, four lanes of the gel are usually required to separate the fragments from a given DNA segment that terminates in A, G, C, or T. Substitution of fluorescent labels for radioisotopes allows the DNA fragments to be detected continuously during electrophoresis. This fluorescent method usually employs four different labels. Using these four labels, all four types of DNA fragments (A, G, C, and T) can be run in one electrophoresis lane. The uncertainty in comparing the label position in four adjacent lanes of the gel is eliminated.

However, both radioactive methods and fluorescent dye labeling methods for DNA sequencing require the use of the time-consuming gel electrophoresis method.

Recently, capillary gel electrophoresis and ultra-thin gel electrophoresis have been pursued to speed up the separation process. Nevertheless, the preparation of the gel and the limitation of the speed for separation by gel makes the possible use of MALDI for DNA sequencing very attractive. It is natural to consider the use of a time-of-flight mass spectrometer for DNA sequencing since the separation time for different sizes of DNA is only a few hundred microseconds or less. A conceptual mass spectra of DNA sequencing is shown in Fig. 5. Special advantages for using MALDI for sequencing include much faster sequencing speed and fewer errors, since the miss band can possibly be figured out by the molecular weight of oligonucleotides.

In order to achieve fast DNA sequencing by laser desorption mass spectrometry, in our opinion, the following three conditions must be met: (1) the size of the DNA fragment to be detected should be larger than 300 nucleotides, which has been routinely sequenced by gel electrophoresis, (2) the sensitivity must be high enough to detect a few femtomole for each DNA segment, and (3) the resolution of mass spectra must be sufficiently high for determining the distribution of DNA with different sizes.

Since the detection of a 500-mer DNA with sensitivity reaching 100 femtomole has been demonstrated, fast DNA sequencing by laser desorption mass spectrometry should become a practical reality if the resolution ($M/\Delta M$) can be improved to be larger than the number of nucleotides each DNA segment contains.

(B) Disease Diagnosis

Due to the potential for very fast DNA sample analysis by MALDI, disease diagnosis and population screening are certainly among the important applications of MALDI. Recently, we tried using MALDI for detection of ΔF508 mutation of the cystic fibrosis gene.[15] Cystic fibrosis (CF) is the most common autosomal recessive genetic disease of Caucasians.[16] The gene responsible for CF is composed of 27 exons spanning 250,000 base pairs (bp) and encodes a protein of 1480 amino acids named the cystic fibrosis transmembrane conductance regulator (CFTR).[17,18] In the North American population, about 70% of the CF carriers have a 3-bp deletion in exon 10, resulting in the loss of a phenylalanine residue at codon 508 (ΔF508).[19,20] Thus, we attempted to demonstrate the use of MALDI for a quick and accurate determination of the CF gene.

To demonstrate detection of a ΔF508 mutation, genomic DNA prepared from a known CF carrier was used as a template to amplify allelic fragments of 98 bp and 95 bp from exon 10 of the CFTR gene with the 16B and 16D primer in the polymerase chain reaction (PCR). Then two oligonucleotide primers CF1 and CF2 were then designed to do nested amplification of a DNA segment spanning the deletion, thus generating a 59 bp and a 56 bp

fragment of the normal CF gene and ΔF508 mutation, respectively. Our results indicate we can distinguish DNA segments from a normal individual, a heterozygous carrier, and an affected CF patient.

(C) DNA Fingerprinting

The application of DNA fingerprinting has very broad usefulness in forensic analysis. DNA fingerprinting was initiated by the use of the restriction fragment length polymorphism (RFLP). Recently, we[21] used MALDI to detect DNA segments produced from restriction enzyme digestion. To explore the potential of mass spectrometric detection of DNA fragments produced by enzyme digestions, 246 bp double-stranded DNA segments were produced from pLB132 with VL3-3 and VL3-6 primer by PCR. The nucleotide sequence is shown in Fig. 6. Cleavage of the amplified DNA with restriction enzyme StyI(C↓CATGG) plus HinfI(G↓ANTC) should generate DNA segments of 87, 78, 37, 22, 14, and 8 bp. A MALDI spectrum for negative ions of the amplified DNA cut with HinfI and StyI is presented in Fig. 7. Results show detected ions corresponding to the single-stranded DNA ions of 22-, 37-, 78-, and 87-mer, respectively. The missing DNA segments of 8 bp and 14 bp were lost during the alcohol precipitation. These results indicate the potential of MALDI for forensic applications. In 1987, Nakamura *et al* [22] found that a variable number of tandem repeats (VNTR) often occured in the alleles. The probability of different individuals having the same number of tandem repeats in several different alleles is very low. Thus, the identification of VNTR from genomic DNA becomes a very reliable method for identification of individuals. Quick and reliable analysis of DNA by MALDI is expected to be extended to detect VNTR in the near future.

V. CONCLUSION

During the past five years, laser desorption mass spectrometry has proved to be very valuable for oligonucleotide analysis. Major progress comprises the discovery of new efficient matrices (such as 3-HPA, PA, and 3-APA), detecting DNA of 500 base pairs, and demonstration of the cystic fibrosis gene.

ACKNOWLEDGEMENT

Research sponsored by the Office of Health and Environmental Research, U. S. Department of Energy under contract DE-AC05-84OR21400 with Martin Marietta Energy Systems, Inc., and by the Director's Fund at Oak Ridge National Laboratory. Preparation of the manuscript by Darlene Holt is also acknowledged.

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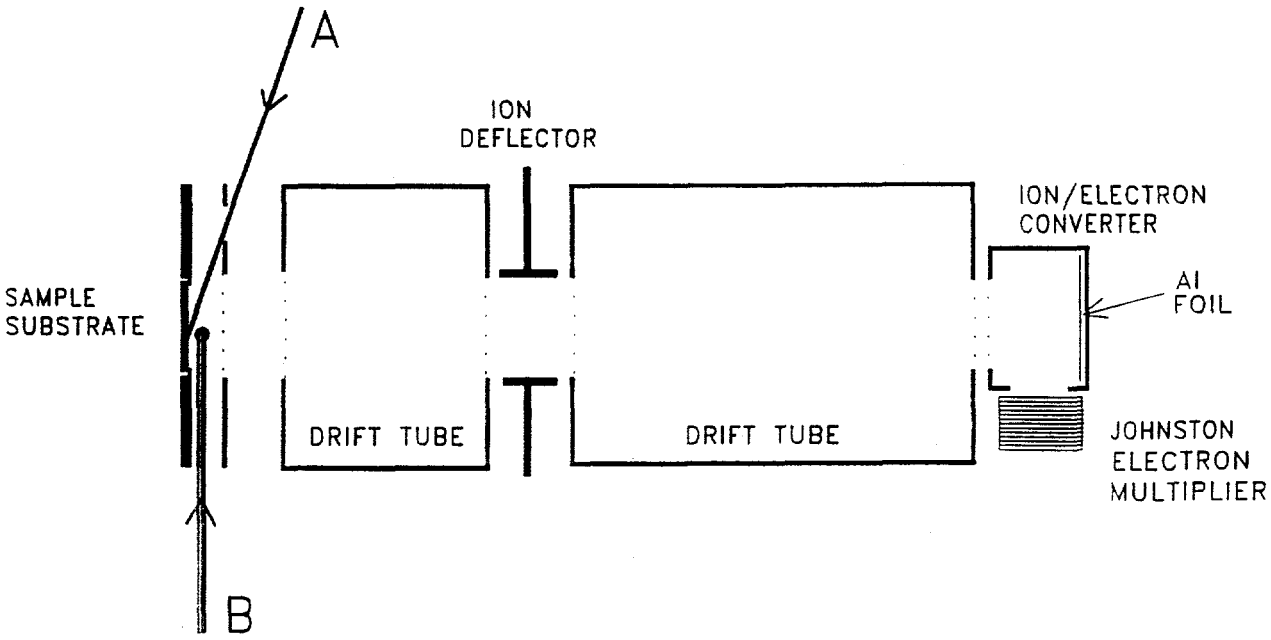

Figure 1 Schematic of a linear time-of-flight mass spectrometer for MALDI research at Oak Ridge National Laboratory.

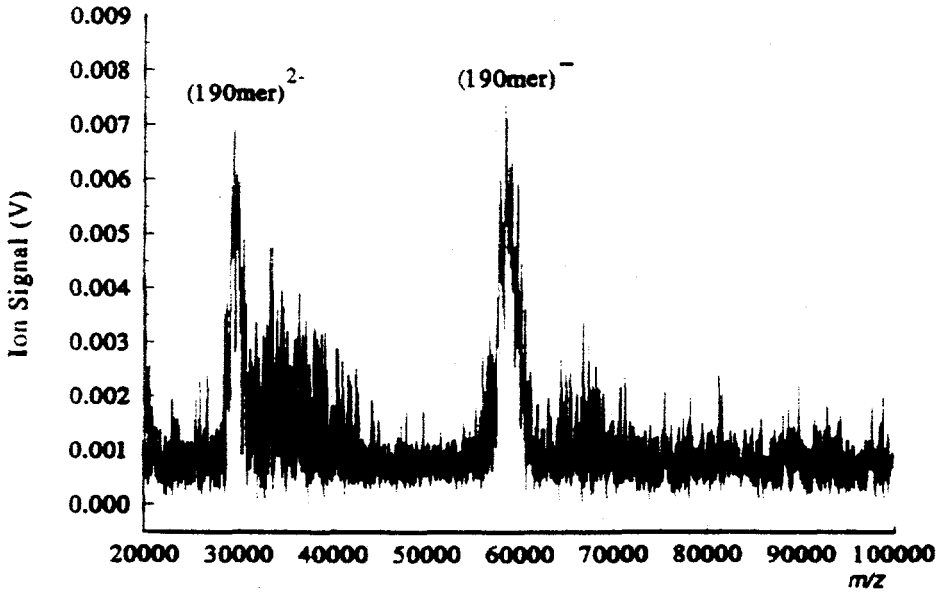

Figure 2 Negative ion mass spectrum of a 190-base-pair DNA obtained from PCR amplification. Total amount of sample loaded was about 500 femtomole. 2,4 μmol picolinic acid was used as the matrix. Laser wavelength was 266 nm and laser fluence was 80 mJ/cm^2.

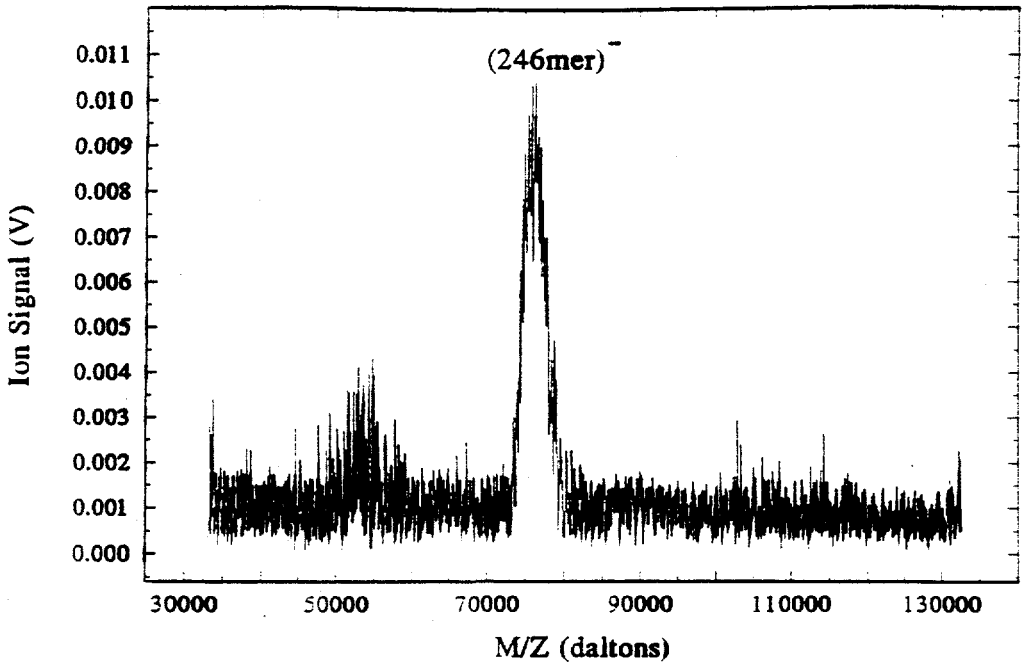

Figure 3 Negative ion spectrum of 246-basepair DNA. The molar ratio of 3-aminopicolinic acid to DNA is 5×10^5:1. Laser wavelength was 266 nm and the laser fluence was 117 mJ/cm^2.

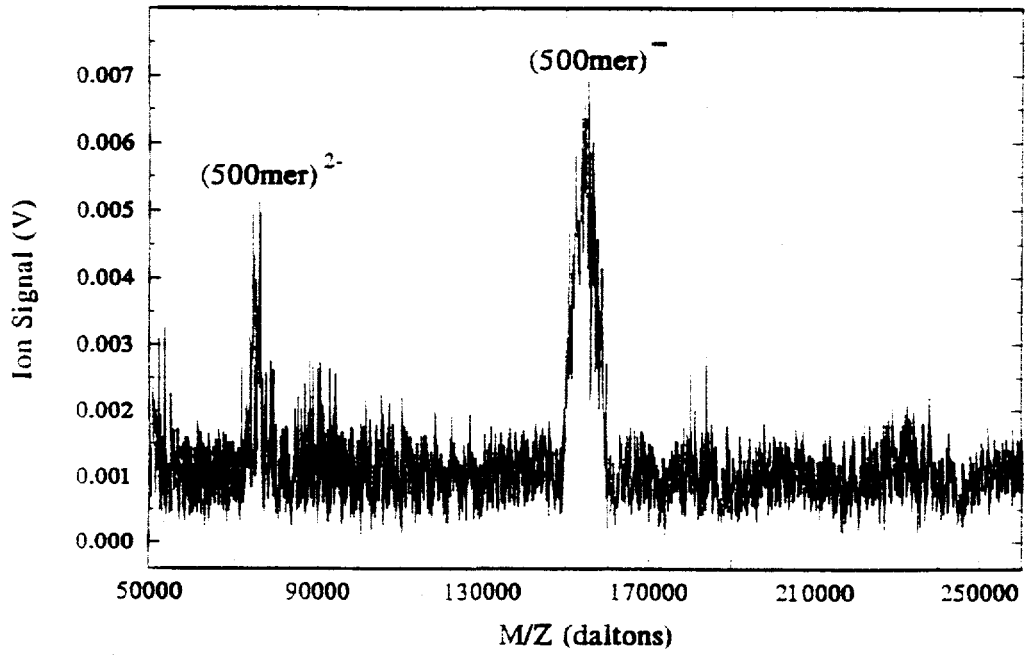

Figure 4 Negative ion spectrum of a 500-basepair DNA amplified from bacteriophage lambda genome. Total amount of DNA loaded was about 2.5 pmol. 2.4 µmol picolinic acid +0.3 µmol 3-HPA was used as a matrix. Laser wavelength was 266 nm and laser fluence was 200 mJ/cm^3. Both singly-charged and doubly-charged ions are observed.

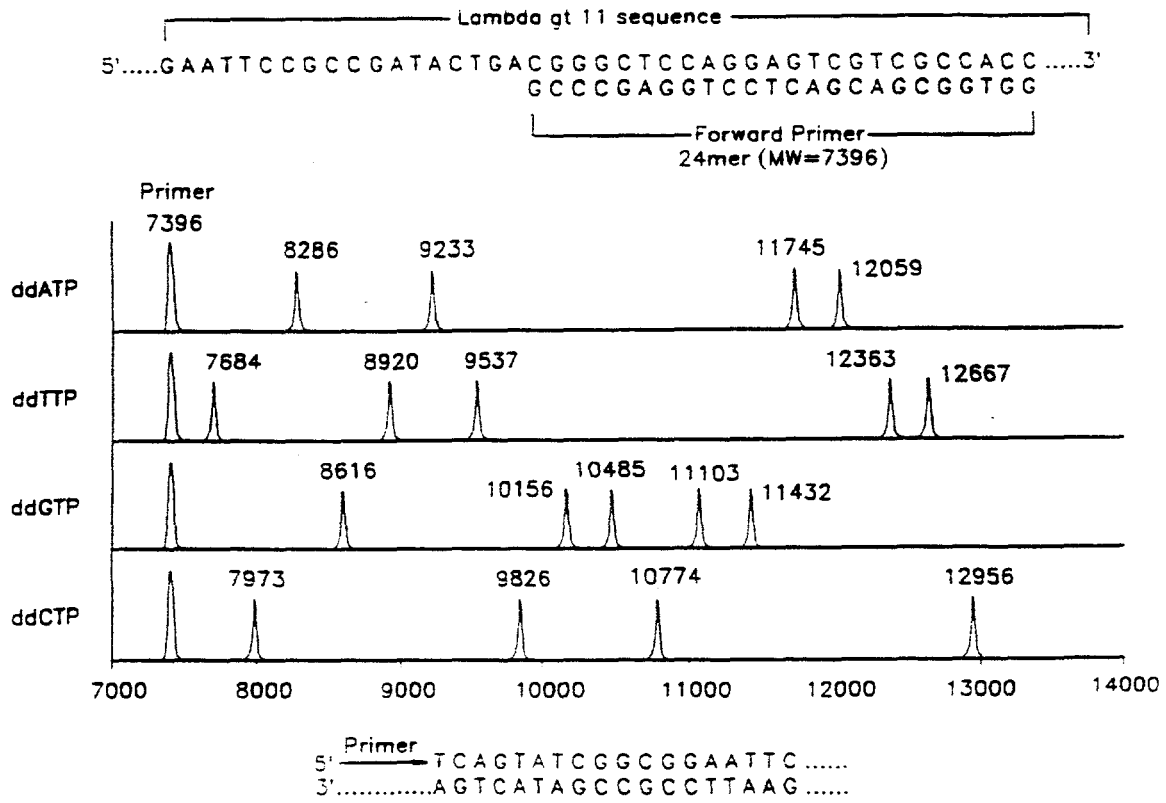

Figure 5 Conceptual mass spectra for DNA sequencing.

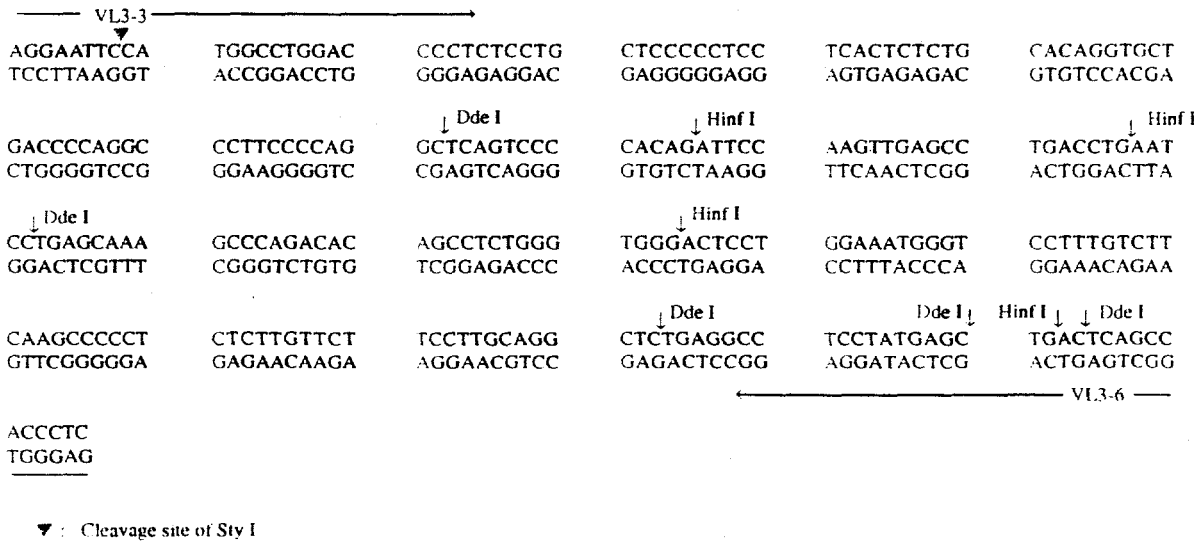

Figure 6 Sequence of DNA designated as pLB 132. The sites for various enzymes to cut are also illustrated.

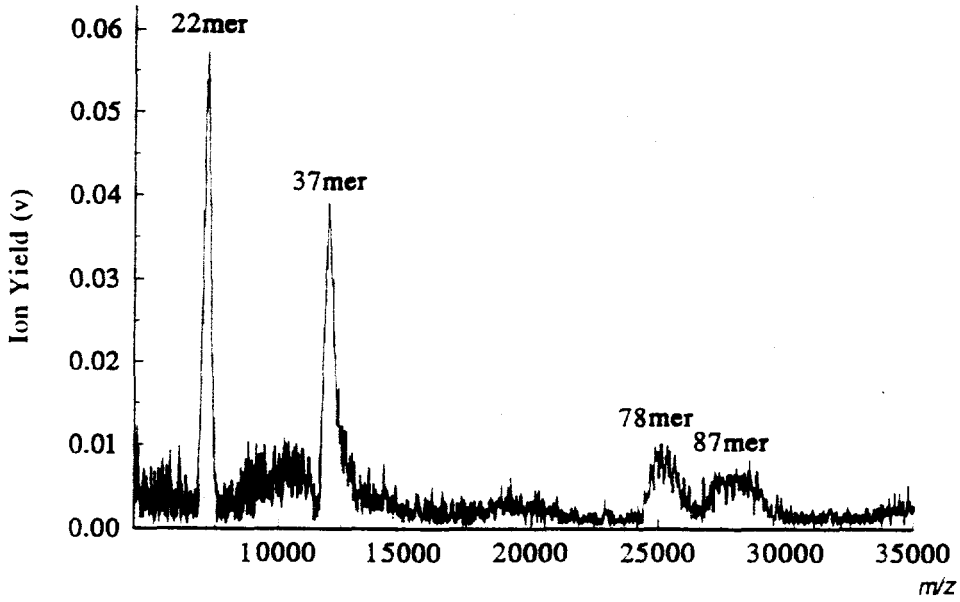

Figure 7 Negative ion spectrum of DNA (pLB 132) cut with HinfI and StyI.