A simple and practical method that prepares high molecular weight DNA ladders

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Abstract. The purpose of the current study was to report a simple and practical method to prepare high molecular weight (mw) DNA ladders. The method involves 1,000-4,000-base pairs (bp) DNA fragments being amplified by polymerase chain reaction (PCR), using λ DNA as a template. The constructed plasmids are digested by restriction endonucleases to produce 5-, 6-, 8- and 10-kb DNA fragments, followed by purification and precipitation with ethanol, and mixed proportionally. The 1,000-4,000-bp DNA fragments were successfully generated by PCR and 5-, 6-, 8- and 10-kb DNA fragments were obtained through the digestion of the plasmids. The bands of the prepared high mw DNA ladder were clear and may aid future molecular biology studies.

Introduction

DNA molecular weight (mw) standard controls of nucleic acids, also known as DNA ladders, are widely used in molecular biology studies to determine the mw or the base pair (bp) length of nucleic acids. The DNA ladder is also used to quantitatively analyze target DNA fragments. At present, there are two ways to prepare a DNA ladder: i) amplification by polymerase chain reaction (PCR) (1,2); and ii) digestion of plasmid DNA by restriction endonucleases (3,4). However, each of these methods have advantages and disadvantages. The former achieves regular bands, but it is difficult to amplify DNA fragments of a high mw. The latter involves the preparation of the DNA ladder mostly from bacteriophages or plasmids, with digestion of the purified DNA by restriction endonucleases, but this process is complex and produces unevenly distributed DNA fragments, particularly for the preparation of large quantities of DNA ladders. The present study describes a method that is based on the combination of PCR amplification and plasmid digestion by restriction endonucleases to prepare a high mw DNA ladder. The results show that the prepared DNA ladder bands were clear, accurate and cheap and may be used as a standard in future molecular biology experiments.

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Materials and methods

PCR primers. According to the λ phage DNA sequence (GenBank accession no. J02459), pairs of 1,000-, 2,000-, 3,000- and 4,000-bp primers were designed with Primer 5.0 software and were obtained from Shanghai Sangon Co., Ltd. (Shanghai, China). The primers are shown in Table I.

PCR amplification. DNA fragments of 1,000-4,000-bp were amplified by PCR. The reaction mixture (100 μl) for each quantitative PCR contained 100 ng template DNA, 0.2 mM dNTP, 10 mM Tris-Cl (pH 8.8), 1.5 mM $MgCl_2$, 50 mM KCl, 0.1 mM of each primer and 2.5 units *Taq* DNA polymerase. All PCR procedures of these DNA fragments were adopted by the improved touch-down PCR method (5) and DNA fragments were amplified using various PCR procedures. The specific PCR procedures were as follows: the PCR conditions of 1,000- and 2,000-bp included a temperature profile of 30 cycles, which included 2 cycles at 95˚C for 40 sec, 60˚C for 50 sec and 72˚C for 50 sec, 2 cycles 95˚C for 40 sec, 59˚C for 50 sec and 72˚C for 50 sec, 2 cycles for every annealing temperature interval one temperature, until 56˚C polishing 20 cycles. The PCR conditions of 3,000- and 4,000-bp included a temperature profile of 30 cycles, which contained 2 cycles at 95˚C for 40 sec, 65˚C for 1 min and 72˚C for 2 min, 2 cycles 95˚C for 40 sec, 64˚C for 1 min and 72˚C for 2 min, 2 cycles for every annealing temperature interval one temperature, until 61˚C polishing 20 cycles.

Construction, purification and identification of plasmids. The construction of high mw plasmids, including 5-, 6-, 8- and 10-kb was carried out as described previously (6), purified with a plasmid DNA purification kit and identified with endonuclease *Hin*dIII. The undigested plasmid of corresponding mw was used as the control and then electrophoresed.

Electrophoresis, purification and sequencing. The PCR products of 1,000- and 2,000-bp were detected by 1% agarose gel electrophoresis at 120 V for 30 min. The PCR products of 3,000- and 4,000-bp, the undigested plasmids and the restriction endonuclease were detected by 0.8% agarose gel at 120 V for 30 min. The PCR products and plasmid DNA were recovered

Table I. Primers used in this study.

Length (bp)	Primers	Melting temperature (˚C)
1,000	5'-GCGGCACGGAGTGGAGCAAG-3'	66.00
	5'-GTTATCGAAATCAGCCACAGGGC-3'	63.68
2,000	5'-GCAGTGACACTGCGCTGGATC-3'	61.90
	5'-GTTATCGAAATCAGCCACAGGGC-3'	63.68
3,000	5'-CAGGCCCGCAGTTATCAGGTC-3'	63.87
	5'-GTTATCGAAATCAGCCACAGGGC-3'	63.68
4,000	5'-CAGCATGCCACGTAAGCGAAACAAAAA-3'	62.00
	5'-CACGGAAAAAGAGACGCAGAAACAGC-3'	63.52

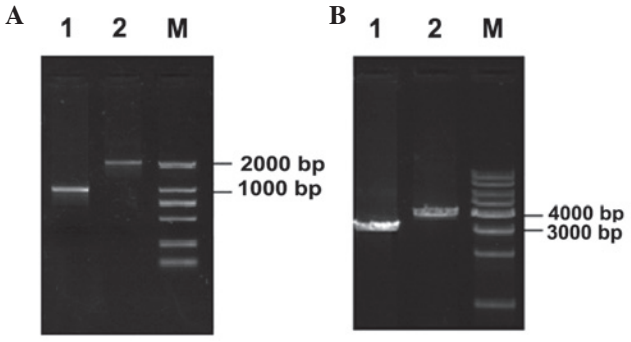

Figure 1. DNA fragments (1,000-4,000-bp) were amplified by PCR. (A) The electrophoresis results of 1,000-bp (lane 1), 2,000-bp (lane 2), DL200 (M). (B) The electrophoresis results of 3,000-bp (lane 1), 4,000-bp (lane 2), 1-kb DNA ladder (M) in (B). PCR, polymerase chain reaction.

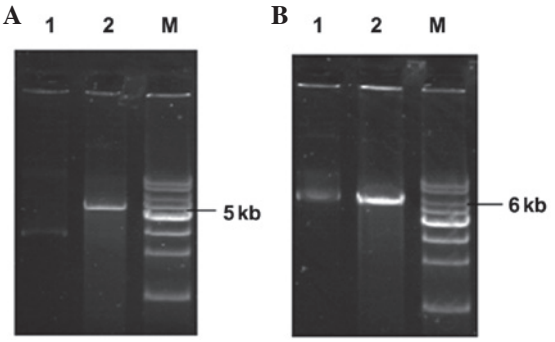

Figure 2. Purification and identification of 5- and 6-kb. (A) The electrophoresis results of 5-kb (lane 1), the linearized strand of 5-kb digested by *Hin*dIII (lane 2), 1-kb DNA ladder (M). (B) The electrophoresis results of 6-kb (lane 1), the linearized strand of 6-kb digested by *Hin*dIII (lane 2), 1-kb DNA ladder (M).

using the agarose gel purification kit according to the manufacturer's instructions, followed by sequencing by Shanghai Sangon Co., Ltd. The generated sequences were compared with BLAST (http://www.ncbi.nlm.nih.gov/ blast/Blast.cgi).

Preparation of a high mw DNA ladder. The PCR products and linear plasmids were extracted by phenol/chloroform and precipitated with ethanol, then dissolved in TE (10 mM Tris-HCl, 1 mM EDTA) buffers and had their UV absorbance analyzed at 260 nm. According to the special proportions, various sizes of DNA fragments were mixed and added to 10X loading buffer and were then ready for use. The prepared DNA ladder was then frozen at -20˚C.

Results

PCR amplification. All DNA fragments of 1,000-4,000-bp were amplified successfully by PCR. As shown in Fig. 1, DNA fragments had high specificity and the sizes of the amplified PCR products were consistent with those expected.

Purification and identification of plasmids. The 4 plasmids and their restriction endonuclease analyses are shown in Figs. 2 and 3. Following the digestion of these constructed vectors by *Hin*dIII, revealing the linearized DNA strands, the results of the restriction were consistent with those expected, the above results indicate that 4 plasmids had been successfully constructed.

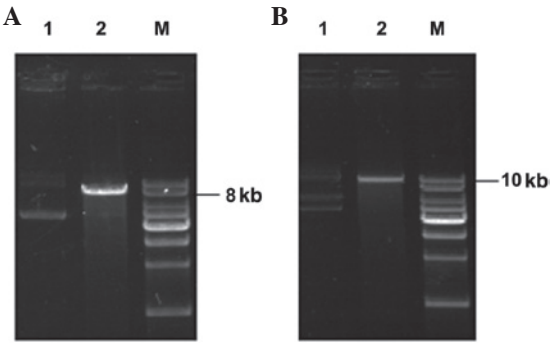

Figure 3. Purification and identification of 8- and 10-kb. (A) The electrophoresis results of 8 kb (lane 1), the linearized strand of 8 kb digested by *Hin*dIII (lane 2), 1-kb DNA ladder (M). (B) The electrophoresis results of 10 kb (lane 1), the linearized strand of 10 kb digested by *Hin*dIII (lane 2), 1-kb DNA ladder (M).

Preparation of high mw DNA ladder. Following extraction, purification and quantity analysis, the PCR products and linear plasmids were mixed according to the special proportions, while the 4,000-bp band was doubled to increase the discrimination effect, then added to 10X loading buffer and was then ready for use. The result of the agarose gel electrophoresis revealed that all the DNA bands were clear and that the ladder was comparable with the commercial version (Fig. 4).

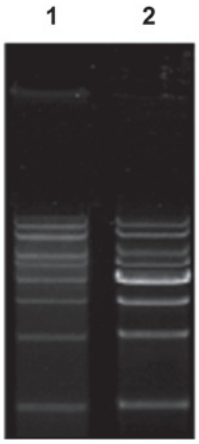

Figure 4. Preparation of high molecular weight DNA ladder. The electrophoresis results of the prepared 1-kb DNA ladder (lane 1), the commercial product of 1-kb DNA ladder (lane 2).

Discussion

The DNA ladder is a widely applied method in electrophoresis and is valuable in molecular biology experiments. It is used to mark the mw of unknown samples in nucleic acid electrophoresis and further aids the judgement of the properties of DNA samples. DNA ladders have been widely applied in such fields as biotechnology, medicine and agriculture, among others, and the market demand is extremely high. PCR was initiated in 1983 by Mullis *et al* (7) as a specific DNA amplification technology and has the advantages of easy operation, high sensitivity and specificity and good selectivity, and has been widely applied in the fields of molecular biology, genetics, biochemistry, genetic engineering and forensics (8-10). Although PCR amplification is convenient for preparing the DNA ladder, conventional PCR is designed to amplify one type of DNA fragment, one tube at a time, while the DNA ladder is an integration of several DNA fragments, which makes the technological process more complex and cumbersome (11). In addition, the technology is only suitable for amplifying small DNA fragments, while the corresponding fragments of a high mw DNA ladder are difficult to obtain. Despite certain large fragments being amplified using high-fidelity DNA polymerase, the specificity is poor and production cost is high and cannot meet the experimental demand. Another routine method of preparing a DNA ladder is using bacteriophages or plasmids which are digested by restriction endonucleases (12,13). This process requires the construction of a series of vectors for preparing the DNA ladder, further digesting the purified DNA with certain restriction endonucleases and subsequently the combinations of DNA fragments that were required are obtained (14,15). This process is laborious, time consuming and material- and equipment-intensive, involving the propagation of the virus or plasmid in the appropriate host organism and the purification and digestion of the genomic or plasmid DNA from the nucleic acids of the host. Since the corresponding restriction enzyme sites of bacteriophages or plasmid DNA are not well balanced, the distribution of the prepared DNA ladder is irregular and the gaps of the DNA bands are variable in size and not convenient (16).

Based on the analysis and research of the advantages and disadvantages of the above two methods, the methods of PCR amplification and plasmid digestion by restriction endonucleases were adopted in the current study to prepare a high mw DNA ladder. In the current study, 1,000-4,000-bp DNA bands were amplified by PCR and the larger DNA bands, including 5-, 6-, 8- and 10-kb, were obtained by restriction endonuclease digestion of the purified plasmids. These DNA fragments were extracted with phenol/chloroform and precipitated with ethanol, further mixed according to the special proportions and added to 10X loading buffer to obtain the high mw DNA ladder. Compared with commercial DNA ladders, this method was simple, practical and low cost, and the DNA bands were clear. It may also be adjusted to any size of mw standard in a certain range according to the experimental requirement and may be used as a standard in molecular experiments. In short, a new method of preparing high mw DNA ladders was developed in this study.

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