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Low Cost DNA Molecular Weight Marker: Primer-Directed Synthesis from pGEM-T Easy Vector

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Abstract

A low cost DNA molecular weight marker was produced by a marker primer-directed synthetic method using pGEM-T Easy vector as the DNA template. Seven primers were used to amplify eight different DNA fragments, which were 150, 300, 375, 500, 700, 1,000, 1,200 and 1,625 bp, from bacterial culture containing pGEM-T Easy vector. Polymerase chain reactions (PCR) for all marker loci required the same optimal annealing temperature, which allowed all the PCR to be completed in a single run. To obtain the molecular weight marker, the PCR product of each locus was mixed together and directly used as marker without any further purification. This custom made molecular weight marker was found to be approximately 17 to 49 times less expensive than other commercial 100 bp DNA ladder markers.

Keywords: DNA molecular weight marker, marker primer-directed synthesis, pGEM-T Easy vector

Introduction

DNA molecular weight marker is a set of known sized DNA fragments, commonly used for comparing the molecular weight of DNA samples on gel electrophoresis. Considering the amount of molecular weight markers used in molecular biology, it can be considered as one of the materials with a significant cumulative cost. Molecular weight markers can be prepared in different ways. One of the original methods is to use restriction enzymes to cleave genomic DNA of bacteriophage lambda [1], or simian virus [2], or the plasmid DNA of bacteria [3] into pieces of different sizes. By this method, the sizes of the DNA fragments are defined by the positions of the restriction sites of the chosen enzymes. Therefore, the molecular weight marker prepared by this method may not produce stepwise sizes like DNA ladder markers. Another method for preparing molecular weight markers is known as a marker primer-directed synthetic method-MPDS [4], which employs polymerase chain reaction (PCR) to amplify DNA fragments of specific sizes. By this method, the DNA ladder marker can be prepared by designing primers corresponding to the sizes of the expected PCR products.

Based on the PCR method, two different molecular weight marker preparations were recently proposed. In 2008, Chang *et al*. [5] suggested a PCR-synthesized marker (PSM) method, by cloning any 100 bp DNA ladder markers into a TA cloning vector such as pGEM-T Easy vector, and using only one pair of vector specific primers to PCR amplify the insert fragment from positive clones. Although this method can avoid the requirements of using many primer pairs to produce all of the desired DNA fragments and the optimization of melting temperature (*Tm*) for each primer pair, the protocol requires intensive cloning at the initial step. The other method of molecular weight marker preparation was suggested by Wang *et al*. [6]. In their study, ten primers were used to amplify ten DNA fragments ranging from 100 - 1,000 bp from the lambda DNA in one multiplex PCR. However, the disadvantage of this method is that it employs

the lambda DNA, which is not commonly used in most laboratories.

pGEM-T Easy vector (Promega) is one of the plasmid vectors that is commonly used in molecular genetics studies. Because it is readily available in many laboratories, pGEM-T Easy vector is considered a good candidate for DNA template in the marker primer-directed synthetic method. The simplicity of bacterial cell structure and plasmid DNA allows the bacterial culture containing the plasmid to be directly put into PCR reactions without having the DNA plasmid purified. In addition, the plasmid can be transformed into bacterial cells and propagated through subculture. This study presents a method of making a molecular weight marker, employing seven primers to amplify eight different DNA fragments of 150, 300, 375, 500, 700, 1,000, 1,200 and 1,625 bp from pGEM-T Easy vector.

Materials and methods

The primers were designed for the pGEM-T vector (Promega) using the vector NTI program (Invitrogen). In addition to the general guidelines [7], the criteria for primer design were that i) the expected amplicons varied in size similar to a 100 bp DNA ladder marker, ii) each amplicon was not located within the cloning site, so that the vectors either with or without DNA insertion can be used as DNA templates, and iii) the least number of primers were used in order to minimize the initial cost. Based on these criteria, seven primers were designed. The location and the sequence of each primer are shown in **Figure 1** and **Table 1**, respectively. All possible combinations of these forward and reverse primers could potentially amplify twelve different DNA fragments varying in size from 75 - 1,625 bp (**Table2**). Of these, eight combinations of primer pairs were chosen for further PCR optimization to produce the molecular weight marker, which offers good general use and clear separation of ladder steps. These include DNA fragments of 150, 300, 375, 500, 700, 1,000, 1,200 and 1,625 bp.

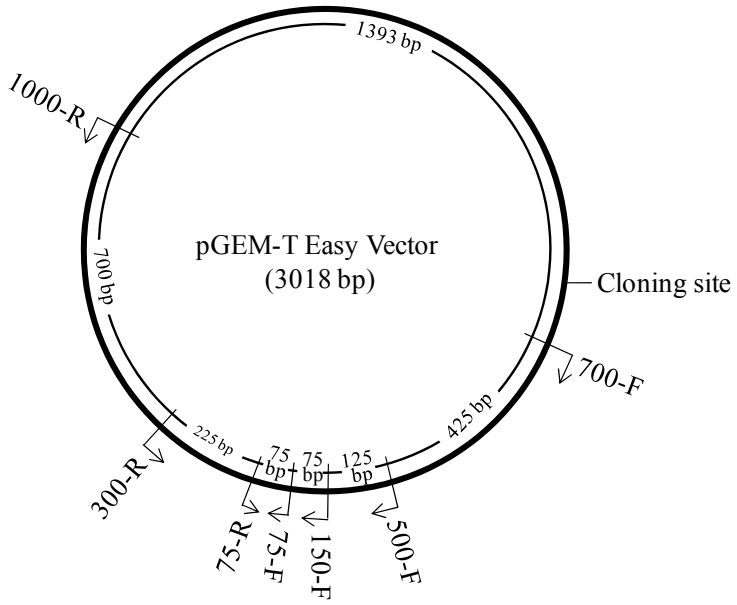

Figure 1 The pGEM-T Easy vector map showing the locations of the primers used in the study.

Table 1 The sequences of the primer used in the study. "F" indicates the forward strand, whereas "R" indicates the reverse strand.

No.	Name	Sequence	*Tm* (°C)
1	75-F	5′- GTAGGTCGTTCGCTCCAAG -3′	62.3
2	75-R	5′- TTACCGGATAAGGCGCAG -3′	59.9
3	150-F	5′- ATACCTGTCCGCCTTTCTCC -3′	62.4
4	300-R	5′- GTGGTTTGTTTGCCGGATCAAG -3′	60.1
5	500-F	5′- GAGCATCACAAAAATCGACGC -3′	58.0
6	700-F	5′- CATAGCTGTTTCCTGTGTG -3′	55.4
7	1,000-R	5′- GGCCAACTTACTTCTGACAACG -3′	60.1

Table 2 All possible combinations of the primer pair and the expected PCR products. Asterisks indicate the products chosen for preparing molecular weight markers in this study.

Combination of primer pair	Size of the expected product (bp)	Combination of primer pair	Size of the expected product (bp)
75-F & 75-R	75	500-F & 75-R	275
75-F & 300-R	300*	500-F & 300-R	500*
75-F & 1,000-R	1,000*	500-F & 1,000-R	1,200*
150-F & 75-R	150*	700-F & 75-R	700*
150-F & 300-R	375*	700-F & 300-R	925
150-F & 1,000-R	1,075	700-F & 1,000-R	1,625*

To prepare the DNA template for PCR, pGEM-T Easy vector was heat shock transformed into *Escherichia coli* Top 10F′ (Invitrogen). A single colony of the transformants was cultured in LB broth with 50 mg/ml ampicillin, incubated at 37 °C with shaking at 150 rpm until OD_{600} = 0.4 - 0.6, and directly subjected into the PCR reactions. The PCR reaction for each DNA fragment was separately performed in 25 µl volume of 10 mM Tris HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.4 mM dNTPs, 10 ρmol of each forward and reverse primer, 1 U *i-Taq*™ DNA Polymerase (Intron Biotechnology), and 1 µl of bacterial culture containing pGEM-T Easy vector.

Since the study aimed to amplify all targeted DNA fragments in a single run, the optimal annealing temperature shared between all loci was determined using temperature gradient ranging from 55 - 60 °C. In addition, the thermal cycle was performed in two sets of 20 consecutive cycles with different extension times. In the first 20 cycles, the extension time was set at 1 min for best amplifying the fragments shorter than 1 kb, and in the latter 20 cycles, the extension time was set at 1.5 min for best amplifying the fragments longer than 1 kb. The thermal cycle was set for initial heat denaturation at 94 °C for 4 min, 20 cycles of 94 ºC for 30 s, 55 - 60 °C for 30 s, and 72 ºC for 1 min, and 20 cycles of 94 ºC for 30 s, 55 - 60 °C for 30 s and 72 ºC for 1.5 min, followed by a final extension at 72 ºC for 10 min.

To optimize the intensity of DNA bands, the PCR products of all loci were mixed together in various ratios, and five microliters of the DNA mixture was directly loaded on a 1.5 % agarose gel containing ethidium bromide. The gel images were captured and analysed using GeneSnap and GeneTools software (Syngene), respectively.

Results and discussion

All eight marker loci were successfully amplified by a wide range of annealing temperature from 55 - 60 ºC (data not shown). The optimal annealing temperature of 57 ºC was used to amplify all eight marker loci in a single run. The molecular weight marker was produced by mixing the PCR products of all loci in an equal volume. However, the 150 bp fragment was added at triple the volume to make the visible band on electrophoresis gels as clear as the other higher molecular weight DNA fragments. In addition, the 500 and 1,000 bp fragments were doubled in order to use as reference bands (**Figure 2**). The optimized protocol for producing this custom molecular weight marker is shown in **Figure 3**.

To produce one lot of the custom molecular weight marker, 12 PCR reactions are required. The 25 µl PCR reaction used in this protocol yielded a total volume of 300 µl of the molecular weight marker. Five microliters of the mixed DNA marker can be directly loaded on an agarose gel without any further DNA purification. Therefore, the total volume of 300 µl mixed product can be used as a molecular weight marker for 60 lanes.

The production of this molecular weight marker from pGEM-T Easy vector was found to be cost effective, with an approximate cost of PCR materials of 6.01 Baht per reaction. Therefore, one lot of the custom molecular weight marker costs 72.12 Baht, which can be used for 60 lanes. This cost is equivalent to 1.20 Baht per lane. Although the market prices may vary, when compared to other commercial 100 bp DNA ladder markers, this custom molecular weight marker costs approximately 17 to 49 times less. Considering the initial cost, the production of this custom molecular weight marker is suggested if more than 500 lanes of molecular weight marker are required.

The advantages of this molecular weight marker production are that the protocol is quick and easy. pGEM-T Easy vector is commonly used in molecular laboratories, and can be propagated through subculture, providing an unlimited DNA template for PCR. The bacterial culture containing the plasmid can be directly subjected to PCR reactions without plasmid purification. In addition, since the PCR reaction for each locus has the same optimal annealing temperature of 57 °C, the amplification for all targeted DNA fragments can be carried out in a single run. To produce a high amount of molecular weight marker, the PCR reactions can be performed in a 96-well format. The mixed PCR products can be immediately used as a molecular weight marker without further purification. However, for long term storage, the mixed PCR products can be purified with phenol/chloroform, precipitated in absolute ethanol, dissolved in a TE buffer, and stored at –20 °C.

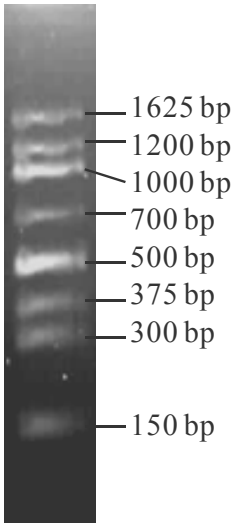

Figure 2 Five microliters of the custom DNA molecular weight marker run on a 1.5 % agarose gel.

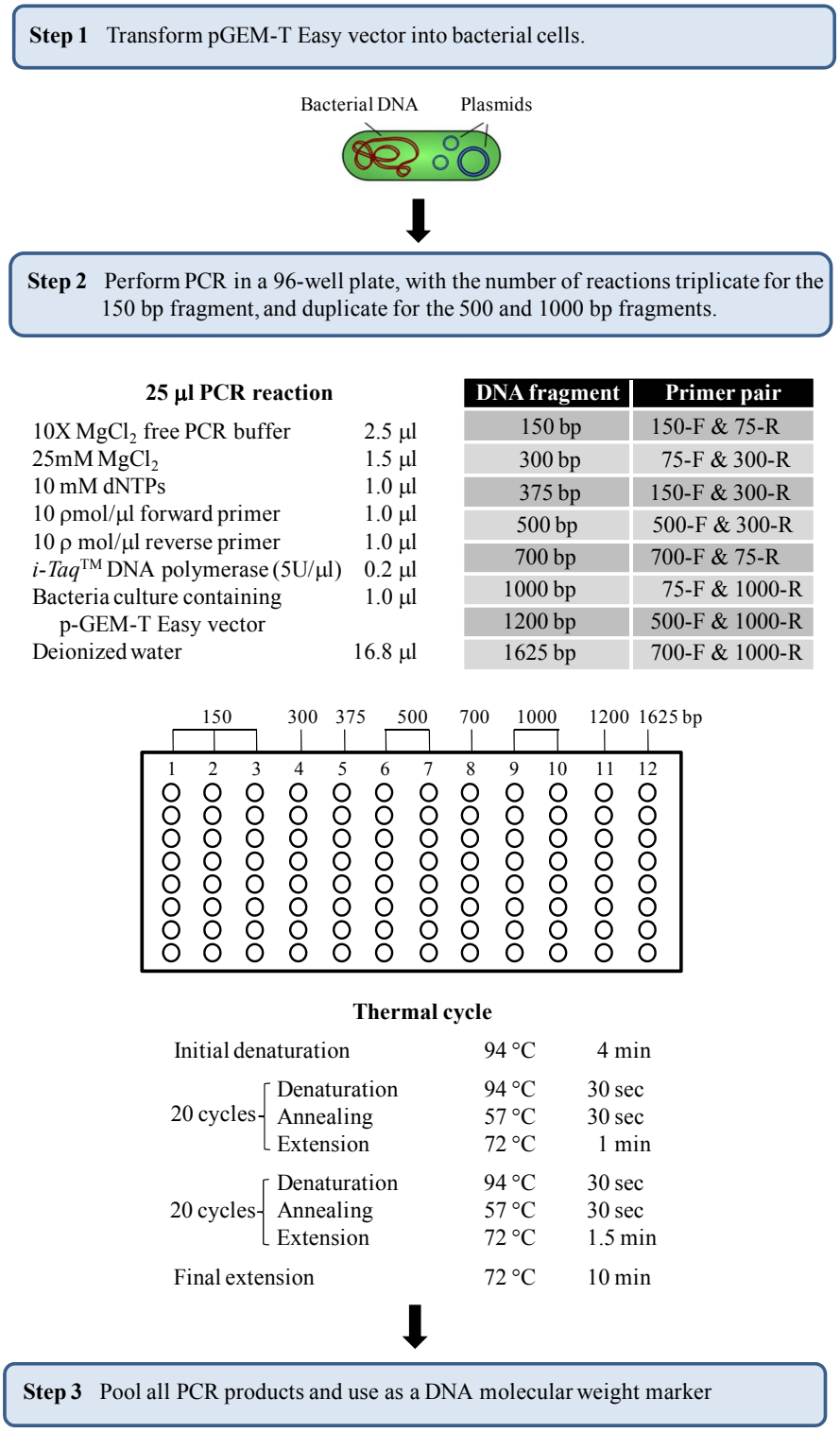

Figure 3 The optimized protocol for preparing the custom DNA molecular weight marker.

Conclusions

The study presents a method of producing a DNA molecular weight marker, employing seven primers to amplify eight different DNA fragments with 150, 300, 375, 500, 700, 1,000, 1,200 and 1,625 bp in length from a pGEM-T Easy vector. The PCR product mixture can be directly loaded on agarose gels and used as a molecular weight marker without further purification. This custom molecular weight marker is cost effective as the unit price was 1.20 Baht per lane, which is approximately 17 to 49 times lower than other commercial 100 bp DNA ladder markers.

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