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# Simple procedure for production of short DNA size markers of 100 to 2000 bp

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## ABSTRACT

DNA size markers (ladder) are essential tools in molecular biology, genetics and biotechnology. In this study, a simple and cost-effective method for laboratory production of DNA ladders is introduced. For this purpose, different sizes of 100 to 2000 bp DNA segments were designed using PCR technique. For producing 14 different gene fragments as DNA molecular weight markers, recombinant plasmid pET28a containing α-amylase gene as a DNA source and one forward and 14 reverse primers were used. The gene fragments containing 100 to 400bp segments with a distance of 50 bp and 400 to 1600 bp segments with a distance of 200 bp as well as 1600 to 2000 bp segments with the distance of 400 bp were generated in a single run of PCR. The present technique could prove to be simple, time saving, inexpensive and good quality approach as compared to the usual DNA ladder preparation procedures. Also, according to the same conditions for designed primers there is a possibility of producing other marker sizes by choosing different types of forward and reverse primers. The PCR product mixture could be directly loaded onto the agarose gel and used as a molecular weight marker without further purification because that was as reliable and uniform as markers from commercial sources. Finally, this marker can be useful for most of molecular biology laboratory techniques.

**Keywords:** Laboratory production; DNA Molecular weight marker; PCR; Molecular biology

## Introduction

In molecular biology, the approximate size of biological macromolecules such as nucleic acids during gel electrophoresis is determined by molecular weight (MW) marker or molecular (DNA) size markers (also referred to as ladder). The DNA, RNA or protein MW markers have known length, which are applied as a reference to estimate the size of unknown molecules. The use of DNA MW marker is integral part of the most genomics experiments such as genetic variation, genetic engineering, PCR and cloning.

Currently, there are a variety of methods for laboratory and commercial manufacturing of DNA MW markers. Digesting DNA with restriction enzymes and PCR based amplification are two main methods that have been used in MW marker generation (1). In

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digestion method, genomic DNA of bacteriophage lambda, or simian virus, or the bacterial plasmid DNA are cleaved into the segments of different sizes (2-4).

In PCR-based amplification method, the desired fragments were amplified from plasmid DNA either by a PCR-synthesized marker (PSM) (1) or maker primer-directed synthesis (MPDS) (5). In PSM method different segments are cloned with the desired size inside a T-vector. Different clones with specific insertions can be generated which are amplifiable only by two universal primers. Whereas, in MPDS method, only one plasmid is used and two specific primers are synthetized for amplification of each fragment. So, we need a number of primers to produce all desired fragments.

Recently, many laboratory protocols describing the preparation of DNA ladders by employing the PCR based technique have been reported (6). PCR is a simple and convenient method performed in a short time. However, preparing DNA ladder using common PCR has been laborious. It is very important to find a new method to produce DNA ladder with a rapid, simple way to reduce costs and improve production (7). In present study, we employed PCR method for the amplification of gene fragments from 100 bp to 2000 bp using fifteen specific primers and recombinant plasmid pET28a, containing α-amylase gene as the DNA template. pET28a vector is readily available in many laboratories. Also, the simplicity of bacterial cells and the plasmids allows the plasmid containing bacterial colonies to be directly employable into PCR reactions without purification. By this method, laboratory personnel can readily synthesize their own DNA ladder, instead of its repeated purchase from commercial sources.

## Materials and methods

*Primer design*

The primers were designed for recombinant plasmid pET28a, containing α-amylase gene, with the help of primer3 software. Out of 14 DNA fragments, 13 fragments were located within amylase gene instead of being present into cloning site, while one fragment was located into vector. Combination of forward and reverse primers produced 100 bp to 2000 bp fragments. Location of each primer is shown in Figure 1. The sequences and Tm of each primer is shown in Table 1 ("F" indicates the forward strand, whereas "R" indicates the reverse strand in primer).

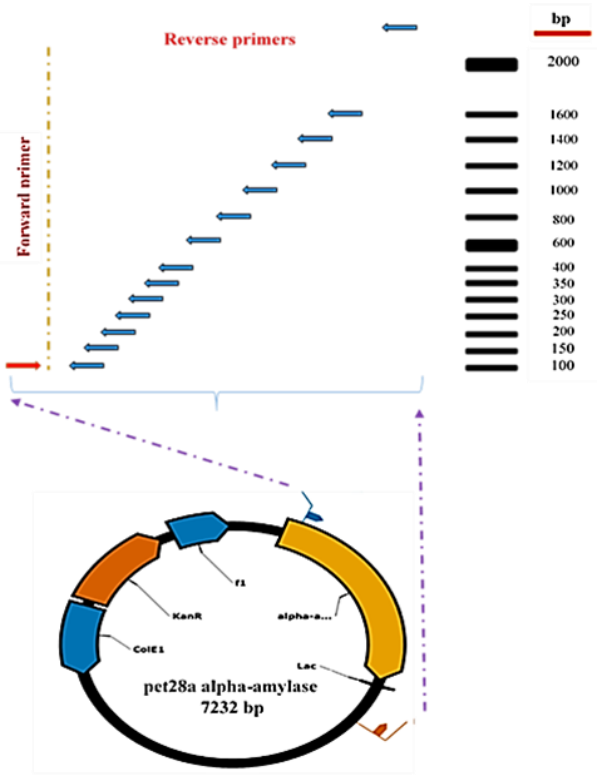


**Figure 1. Recombinant pET28a vector map showing the locations of primers, used in this study**

**Table 1. Sequence of primers used in this study**

| Name | Reverse primers | Fragment size |
|---|---|---|
| **Forward Primer** CTGGCTTTACCAGCCGACCT3' | 5'AACCTTCACACCATATTCTTCAGC3' | 100 |
| | 5'CGCAGCATAGTCACTGGTTGTG3' | 150 |
| | 5'TGTGTTTCCATGCGTCCAAT3' | 200 |
| | 5'GAGCAATGAATTCTGCGTAACA3' | 250 |
| | 5'CGTTTCAGATAGGACTGTACTTGTGT3' | 300 |
| | 5'TCATAGCGAAATCCGTCTGC3' | 350 |
| | 5'GGCTGCCGTAATTCCCATC3' | 400 |
| | 5'GGCTGACACATCAGATGCATAAT3' | 600 |
| | 5'GCGATCGCCTATTTGGGTTT3' | 800 |
| | 5'TGCCATCAGGTAATTTCGTTG3' | 100 |
| | 5'ACATCTGCACGCAGGGTGAT3' | 1200 |
| | 5'CAATTGTTTTGGCCGCAGAC3' | 1400 |
| | 5'GCACTTGGGCACTGCCATTATT3' | 1600 |
| | 5'GCCATACCGCGAAAGGTTTT3' | 2000 |

*PCR reaction and preparation of DNA MW marker*

To prepare the DNA template for PCR, recombinant plasmid pET28a was transformed into *Escherichia coli*, using heat shock method and cultured in LB agar plate containing Kanamycin antibiotic (50 µg/ml). A single colony of transformants was directly subjected into the PCR reactions. The PCR reaction for each DNA fragment was separately performed in 25 µl volume of 10 µl master mix (ThermoScientific), containing 10 pmol of each forward and reverse primers, 1 unit of *Taq* polymerase (CinnaGen) and single colony of transformed bacteria. For amplifying all DNA fragments in a single run, optimal annealing temperature, shared between all loci, was determined using temperature gradient, ranging from 54-60°C. PCR cycling conditions were as follows: for initial denaturation, 94°C for 5 min; 35 cycles of 94°C for 1 min, 55-60°C for 30 sec and 72°C for 1 min, followed by a final extension at 72°C for 5 min. A total of 5 µl of each PCR product was loaded on a 1.5% agarose gel and the length of fragments were estimated by comparing 100 bp DNA size marker (SinaClon). The fragments were condensed using ethanol precipitation in order to increase their quality. Finally, the concentration of each fragment was analyzed using UV absorbance under 260/280 nm. The marker was frozen and kept at -20°C for further application.

## Results

A schematic image of amylase gene and the location of primers in gene and plasmid are shown in Figure 1. All fragments of 100 to 2000 bp were successfully amplified with single run PCR using the optimization process in temperature profile (Fig. 2). In addition to optimized reaction system and annealing temperatures for avoiding nonspecific reactions, we used ethanol precipitation method for the condensation of fragments and improved the quality of DNA, stacked into the agarose gel. After that, the concentration of each fragment was determined by reading the absorbance by a spectrophotometer. The present technique is proved to be simple, time-saving, inexpensive and a good quality approach at 260/280 nm. Figure 3 shows the condensed fragments of DNA ladder and their concentrations.

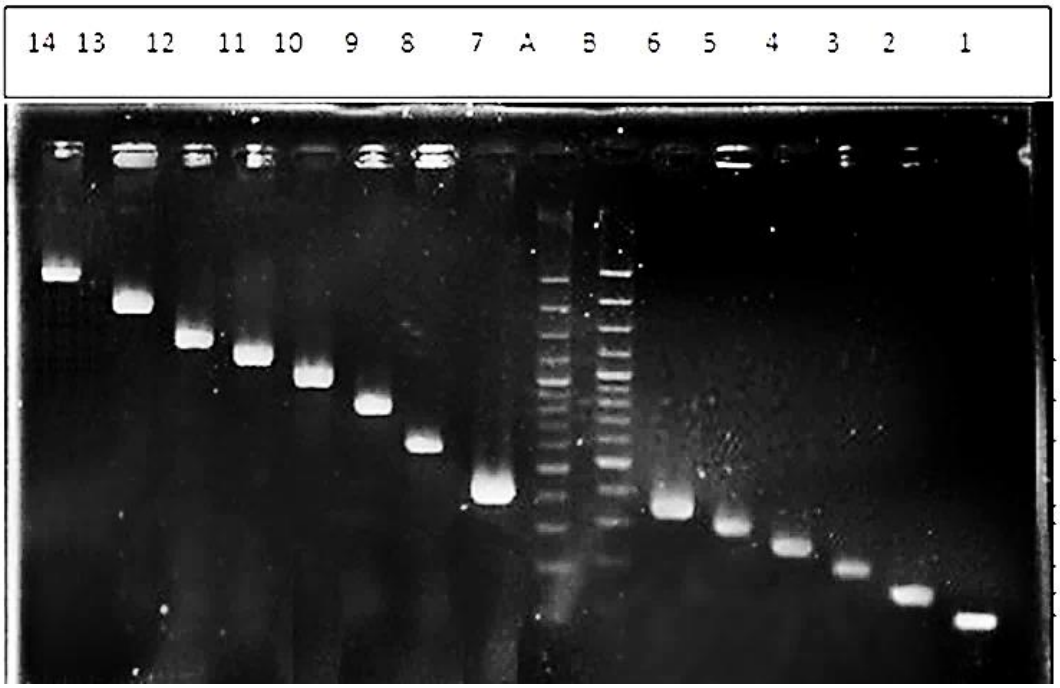


**Figure 2. A volume of 5 µl of the DNA marker loaded onto 1.5% agarose gel. Lanes 1 to 14, represent molecular weights of: 100, 150, 200, 250, 300, 350, 400, 600, 800, 1000, 1200, 1400, 1600 and 2000 bp, respectively. (A) and (B) represent molecular weight marker (Thermo Scientific, SM0321).**

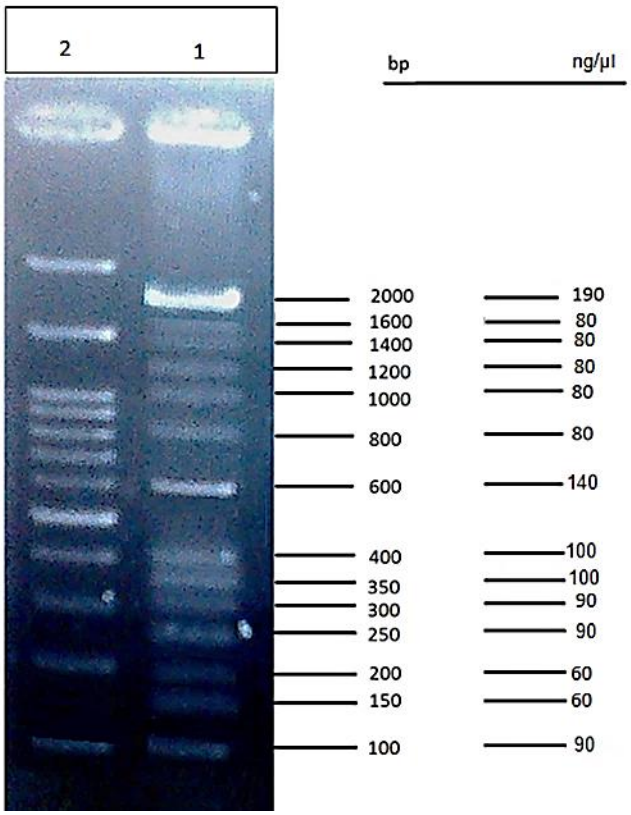


**Figure 3. Gel electrophoresis of DNA fragments condensed with different concentrations; Lane 1, the DNA molecular weight with ng/5 µl concentration, Lane 2, 100 bp DNA ladder (SinaClon, PR911653).**

## Discussion

The novel method of primer design used in this study has a number of benefits as compared to the previous methods. The advantages of this method lies in producing fragments with precise dimensions through PCR, lack of digestion enzymes, the use of a single source gene as a template, high quality without any byproduct, resulting in economic costs and saving time. The parameters such as 3'-stability, duplex formation and false priming were considered for designing of every primer (forward and reverse). Also, after optimizing the quality of this marker, there is no need to use the purification kit, and only extensive replication and condensation could raise the quality of each sample band on agarose gel. Enzyme digestion of phage or viral vectors is one of the conventional primer designing techniques but for producing marker with precise dimensions and identification potential, phages and plasmids cannot be used (8). Therefore, design and construction of genetically engineered plasmids were carried out using restriction enzyme sites at specific regions, and PCR method was used to produce each fragment (8). Despite the fact that enzyme digestion method seems to be economic but in practice it usually has the disadvantage of using several restriction endo-nucleases and high cost (8, 9). Also, despite of cloning the appropriate repeating elements in plasmid backbone, engineered plasmid was not considered as DNA template. Therefore, after digestion of the plas-mid using specific restriction enzymes, interference between the plasmid backbone and other fragments must be omitted by purification steps (10). Polyarush et al. (4) and Cooney (2) separately reported the DNA markers, which were built by enzyme digestion of *E.coli* plasmid and genomic DNA of bacteriophages. Disadvantage of their work was non-uniform banding pattern in electrophoresis as well as the size and number of bands depending on the frequency of recognition site of restriction enzymes. Another disadvantage of digestion of synthetic vector is the weak production efficiency of small DNA fragments. Also, constructing synthetic plasmids for DNA size marker production is not a satisfying procedure in many laboratories (11).

Instead, due to convenient manipulation of PCR reaction, it is a common choice for construction of small DNA fragments for laboratory and industrial purposes (12). Wang et al. (7) have used PCR to build DNA ladder. The primers were designed, based on phage lambda sequence, and the fragments were amplified by multiplex PCR, resulting in the design of DNA ladders with precise dimensions. Hyman (13) cloned several gene copies in pUC18 plasmid to produce 100 bp ladders. Disadvantage of this method was the production of unwanted fragment sizes (13).

## CONCLUSION

In present study, simplified primer-directed PCR based method was used to prepare 100 bp-2000 bp molecular weight marker, using 15 primers in a single PCR run. Total volume of PCR reactions was 350 µl. The total volume of reaction mixture reached to 100 µl and 5 µl of the mixed DNA marker could be directly loaded onto the agarose gel. Therefore, this total volume of product could be used for 20 lanes. Our molecular weight marker had 14 bands of increasing size. There is no need to purify PCR products and the amplified fragments could be directly used in gel electrophoresis. The molecular weight marker, produced in our laboratory according to the existing facilities, was affordable rather than be purchased from commercial sources.

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