Evaluation of the methods used in the production of 100 bp DNA Ladders

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ABSTRACT

Determination of nucleic acid size is a basic need in molecular biology. DNA ladders used for this purpose contain a mixtureof double-stranded DNA products with known sizes, increasing in size at regular intervals. During electrophoresis, these DNA fragments separate according to their mobility in the electric field and they form bands on the gel. The resulting bands are used as references to estimate the size of DNA fragments of unknown size. Three most common methods used in the preparation of DNA ladders include ligation, amplification by PCR and restriction enzyme digestion. Ligation method is a very time-consuming and costly method. Restriction digestion enzymes utilizes bacteriophage and plasmid DNA and is good for the production of large size DNA fragments. The target DNA region can be amplified in a very short time and obtained in high amounts by PCR method. This method is advantageous for the production of smaller size DNA fragments. In the PCR method, a target sequence is cloned into a plasmid. Primer sets are designed and optimized to produce fragments with different sizes in the ladder. Amplification is performed in multiplex PCR approach, however this step needs careful optimization of several parameters including primer sequences, annealing temperature and primer concentration to prevent primer dimer formation and secondary structures. In addition, touch-down PCR may be used to minimize non-specific primer binding during PCR amplification. In this analysis, we focus on cost-effective and fast PCR-based production methods of 100 bp DNA ladders.

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INTRODUCTION

DNA or other molecules' ladders are frequently used as a reference for size determination of molecules such as DNA, RNA or protein in agarose or polyacrylamide gel electrophoresis. There are three different methods commonly used in the preparation of DNA Ladders. These are ligation (Singer, 1998), amplification by PCR (Wang et al., 2010; Wu & Ye, 2011), and cutting with restriction enzymes (Chen et al., 2009; Dodgson et al., 1997). Ligation is based on the principle of covalent joining of phosphodiester bonds that form DNA fragments of different sizes. However, it is a time-consuming and costly method

Figure 1. Schematic representation of ligation-based DNA Ladder production.

Sources such as lambda phage, simian virus 40, Bacillus anthracis strain and plasmid are routinely used in DNA ladder production in cutting processes with restriction enzymes. The method with restriction enzymes requires the reproduction of the virus or plasmid in the appropriate host organism, purification of the viral or plasmid DNA by separating it from the nucleic acids of the host, digesting the purified DNA with restriction enzymes, and finally purification of the obtained fragments. DNA ladder lengths obtained from these natural sources are controlled by restriction enzyme cleavage sites and vary. While this method is disadvantageous in terms of time and cost when used for the production of small molecular weight DNA fragments, it is advantageous over other methods when it comes to generating larger molecular weight DNA fragments.

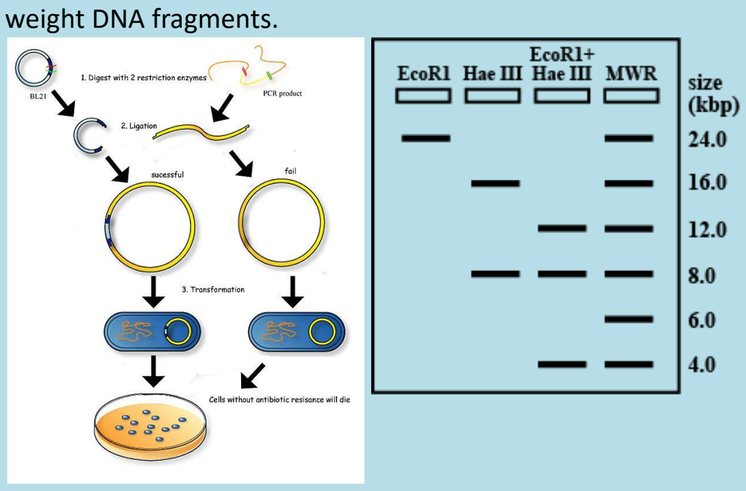

Figure 2. Schematic representation of the cutting method with the restriction enzyme

Although there are different methods in DNA ladder production, there is no easy and flexible technique that enables the production of DNA ladder by self efforts of researchers in the laboratory. Recently, some strategies based on different PCR techniques have been reported in the literature (Abbasian et al., 2015; Amills et al., 1996; Chang et al., 2008; Yasser, 2006). The PCR method is a simple, effective and useful method. Thanks to this method, the target DNA region can be amplified in a very short time and a high amount can be obtained. In addition, many commercially available DNA ladders are available in the market at relatively high prices. Since production with PCR amplification methods is less costly, DNA ladders produced by this method can be found in the market at lower prices than their counterparts. However, it is somewhat difficult to amplify DNA fragments with long dimensions by the PCR amplification method. 3 different PCR methods are mostly used in the production of DNA ladders and reduction of non-specific products during this process. These are Hot-Start PCR, Touchdown PCR and multiplex PCR. These methods have their own advantages and disadvantages. In this review, it was aimed to evaluate these different PCR amplification techniques that can be used in the production of 100 bp DNA ladder

METHODS

Amplification by Hot-Start PCR

The Hot-Start PCR method is a method based on the fact that the enzyme stays away from other PCR components in the presence of various chemicals or modifications and the reaction starts only after a certain temperature. This PCR method, which reduces non-specific bindings and can therefore be used in the production of DNA Ladder, can be applied in various ways. One of the ways to inactivate Taq DNA polymerase up to a certain temperature is to use enzyme-linked antibodies. This temperature-sensitive antibody-enzyme structure breaks down above 70°C and the reaction begins. Another option is to put wax between Taq polymerase and other PCR components, keeping them separate until the temperature rises. Additionally, freezing of PCR components and adding Taq polymerase on top of frozen components is offered as an option. Finally, the option of adding Taq polymerase to the reaction in the device can be implemented. However, this method, which aims to prevent non-specific binding, has disadvantages such as the expensive use of antibodies and wax, and the risk of cross-contamination by adding the enzyme later while the device is operating.

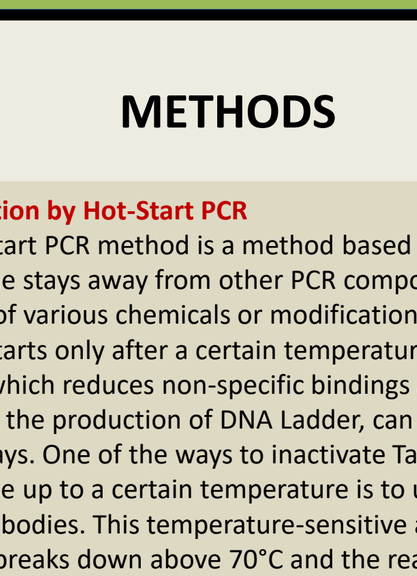

Figure 3. Schematic representation of the amplification method by Hot-Start PCR

Amplification by Touchdown PCR

It is a PCR modification that allows to reduce unwanted amplifications and achieve high specificity by sequentially lowering the annealing temperature after each PCR cycle. In this method, the annealing temperature is chosen 10°C higher than the Tm temperature of the primers. Thus, primers can only bind to their specific and complementary sequences. After each cycle, the temperature is lowered by 1°C. After a certain number of cycles (usually 10 cycles), PCR continues at a stable annealing temperature.

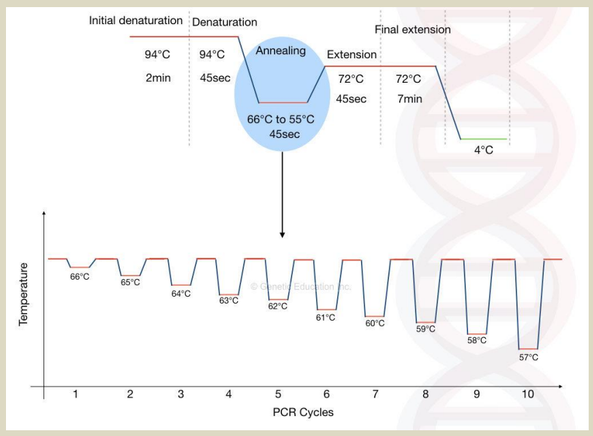

Figure 4. Graphical representation of the sequential reduction of annealing temperature in the amplification method by touchdown PCR.

Amplification by Multiplex PCR

Multiplex PCR is based on the method of amplifying one or more DNA target sites in a single reaction using multiple primer sets. It is a fast method, but it has several disadvantages. The most important of these disadvantages are the non-specific binding of primers to unwanted sites and the possibility of forming dimers.

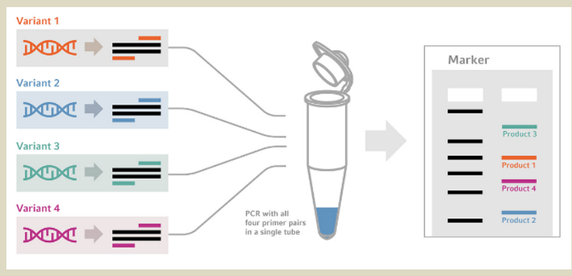

Figure 5. Schematic representation of the multiplex PCR technique.

CONCLUSIONS

The use of these 3 PCR methods together provides advantages in terms of preventing non-specific binding and reducing the risk of secondary structures formation, but also increases the cost.

In the production of a DNA ladder, it is of great importance having amplicons of the right size, preventing unwanted binding and being advantageous in terms of product quality. Preference of the use of different PCR methods described above in DNA ladder production depends on the purpose and needs of the manufacturer.

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