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Research Note

Modified gel preparation for distinct DNA fragment analysis in agarose gel electrophoresis

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Abstract. Agarose gel electrophoresis is the standard method that is used to separate, identify, and purify DNA fragments. However, this method is time-consuming and capable of separating limited range of fragments. A new technique of gel preparation was developed to improve the DNA fragment analysis via electrophoresis.

Typically, agarose gels are used to separate larger fragments of DNA (greater than 200 bp) and polyacrylamide gels are used for small fragments (less than 200 bp). The distance between two DNA fragments of different sizes can be determined based on the concentration of the agarose matrix (Sambrook *et al.*, 1989). The relative mobility of DNA depends primarily on the agarose concentration in the gel, strength of the applied current, the ionic strength of the buffers, and the conformation of DNA fragments. DNA molecules migrate through the small pores that formed within the solidified agarose gel. In general, smaller molecules move faster and migrate farther than the longer ones as small molecules have lesser friction to move towards the positive electrode. Low concentrations of agarose provide better resolution for large fragments by providing greater separation between bands that are close in size. High gel concentrations, on the other hand, reduce the migration speed of the long fragments while facilitating better separation of small DNA fragments. However, large fragments migrate proportionately faster than small fragments as the voltage applied to a gel is increased. DNA possesses a consistent charge to mass ratio, thus the size of DNA molecules is the main factor influencing migration through a gel matrix. Despite the fact that application of high voltages may enhance the migration speed of DNA fragments, it also decreases the resolution of separation (above about 5 to 8 V/cm). For that reason, the best resolution of fragments larger than 2 kb is attained by applying no more than 5 V/cm to the gel.

Generally, agarose gels are cast at a single concentration by melting the agarose in the presence of a desired buffer (Tris-acetate-EDTA or Tris-borate-EDTA) that establishes a pH and provides ions to support conductivity. As for visibility, ethidium bromide, a fluorescent dye that intercalates between bases of nucleic acids, allows the detection of DNA fragments in gels. The binding of ethidium bromide to DNA reduces the electrophoretic mobility by extending the length of linear DNA and making the nicked circular DNA molecules more rigid.

Restriction enzyme analysis of chromosomal DNA as a means of

characterization and diagnosis of bacterial infections has been a valuable tool for decades (Marshall *et al.*, 1981, 1984; O Hara *et al.*, 1985; Venkatesha & Ramadass, 2001). The bacterial restriction endonuclease DNA analysis (BRENDA) has been used for decades as the technique is reproducible and inexpensive. Moreover, BRENDA does not require critical optimization and sophisticated equipment in order to analyse the banding patterns. Nevertheless, improvements are needed for more rapid diagnosis. Although isolation procedures for bacterial chromosomal DNA are becoming more advanced, the process of gel electrophoresis remains unchanged. Traditionally, BRENDA is performed via single concentration of agarose gel electrophoresis and the process to achieve well separated DNA fragments is time-consuming (Venkatesha & Ramadass, 2001). Thus, a new technique of gel preparation involving two different concentrations of agarose, was developed with improved capability of resolving DNA fragments yet requiring a shorter running duration. Combination of high and low agarose concentrations would facilitate distinct migration of both smaller and larger fragments of DNA at a consistent voltage (4.5 V/cm). In addition, this newly developed method enables the observation of distinct banding patterns without the need of preparing large gels which are difficult to handle.

The newly developed technique on agarose gel preparation has proven to be able to provide good separation for both large and small DNA fragments at high voltage (4.5 V/cm). Figure 1 illustrates the new technique of gel preparation for distinct DNA fragments analysis. By having 2% agarose concentration as standard, smaller fragments were able to migrate faster and farther than the larger fragments to get into the lower agarose concentration. The high concentration of agarose (2%) was determined via optimization. This part of gel basically serves as a sieve to facilitate the migration of small fragments into the lower agarose concentration while slowing down the larger fragments. This method promotes distinctive separation of DNA fragments of all sizes and avoids bias.

Figure 2 shows the results of agarose gel electrophoresis on two different gel preparations performed under analogous conditions (gel size, voltage and time). Gel A was prepared using a single agarose concentration (0.8%) to represent the

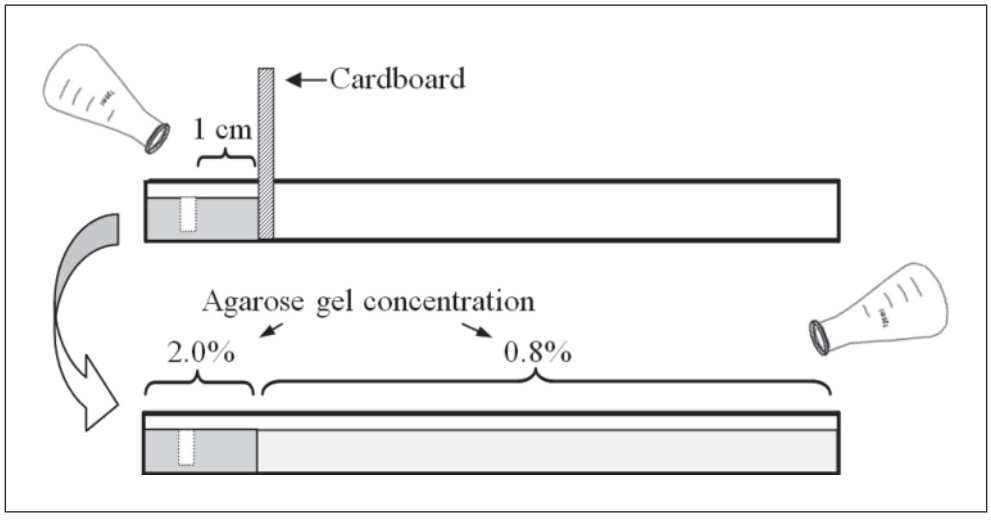

Figure 1. New technique of agarose gel preparation for distinct DNA fragments analysis in agarose gel electrophoresis. The first part of the gel (2.0%) is allowed to solidify in the gel cast before the cardboard is removed. Second part of the gel (0.8%) will subsequently cover the remaining surface of the gel cast

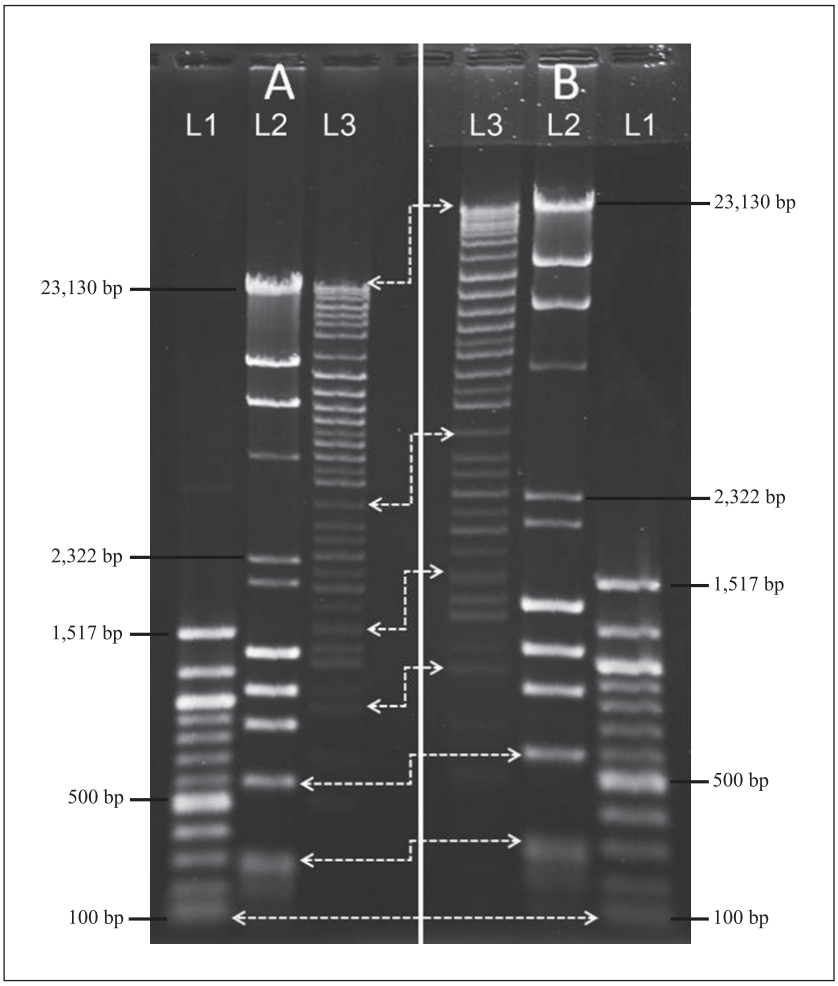

Figure 2. Agarose gel electrophoresis showing migration of DNA fragments in conventional method (A – 0.8% gel) and newly developed method (B – 2% + 0.8% gel). Lane 1: 1kb DNA marker; Lane 2: ˆ DNA *Hin*d III and ΦX174 DNA - *Hae* III marker; Lane 3: Analytical marker DNA wide range

traditional method of gel preparation for BRENDA while Gel B represents the new technique which consists of two different gel concentrations (2% and 0.8%). The electrophoresis process for both gel preparations were conducted at 4.5 V/cm for 1.7 hours. As can be observed, though the migration rate of the smallest fragment (100 bp) was similar in both gels, the separation of subsequent DNA fragments in Gel B improved regardless of the fragment size. This indicated that the dual concentration gel was able to facilitate the migration of smaller DNA fragments efficiently as well as to provide distinctive separation of large DNA fragments without bias. Besides, higher voltage could be introduced via this method to shorten the electrophoresis time required. Conversely, the traditional method of gel preparation in BRENDA requires large gels to obtain a good resolution for both large and small DNA fragments. Large gels are generally hard to handle and require lower voltage to avoid over-heating. Hence, in traditional way of BRENDA, achieving a fine distribution of banding patterns is time-consuming. With this newer method, a good resolution could be achieved for both the short and long fragments within a

comparatively short period of running time without resorting to large agarose gels.

Undeniably, there is always a need for rapid and reliable diagnostic techniques. Various attempts are made to improve the current electrophoresis method. By introducing two different agarose concentrations, electrophoresis can be conducted rapidly with a high voltage while providing a better resolution of DNA fragments. The only drawback of this suggested technique is perhaps the method of preparing a gel with two agarose concentrations which might be slightly time-consuming and tedious as compared to the traditional method of gel preparation for BRENDA. However, the new technology described in the current study not only was able to provide similar resolution of small fragments as can be achieved by the traditional BRENDA, yet a remarkably better resolution for larger DNA fragments. Hence, this new technique is useful in the rapid analysis of an array of small to large DNA fragments.

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