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Novel Application of Reversed Gel Electrophoresis: Reuse of DNA Molecular Weight Marker

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ABSTRACT

Gel electrophoresis is used in nucleic acid analysis and makes use of DNA/RNA molecular weight marker for size comparison. These size markers are used only once per electrophoresis and finally discarded along with the gel. In the present study, a reversed current is applied in agarose gel electrophoresis to reuse the DNA molecular weight marker, thus cutting down the cost involved in molecular experiments.

Key Words: Agarose gel electrophoresis, DNA ladder, Nucleic acid

INTRODUCTION

Agarose gel electrophoresis is a method for separating nucleic acids based on size by comparing DNA or RNA against standard molecular weight marker[1]. These markers are also called as nucleic acid ladders which contain various DNA or RNA of specific length. Agarose gel electrophoresis, which is mainly used for restriction fragment analysis and PCR product analysis, uses DNA ladder for size comparison. The agarose gel is loaded with DNA test sample and DNA molecular weight marker and when electric current is passed DNA moves towards anode. After the completion of electrophoresis the aligned position of DNA test sample is compared with the resolved DNA ladder and the size of DNA in test sample is determined. The resolved DNA ladder is not reused for another electrophoresis experiment. Reversed electrophoresis had been used to purify protein from polyacrylamide gel[2]. Rapid methods for isolation of peptides from sodium dodecyl sulphate (SDS) containing polyacrylamide gels have been applied[3] and been used for purification of chymotrypsin inhibitor isolforms[4].

In agarose gel electrophoresis, after the separation of DNA, the sample well corresponding to resolved DNA ladder is sealed with agarose. By changing the polarity of electrodes or placing the agarose gel with the wells facing towards anode results in movement of resolved DNA ladder converging back as a single band in the sealed well of agarose gel. After 15-20 minutes of reversed electrophoresis, the gel is kept for 20 minutes in a vessel containing 0.5 µg/ml ethidium bromide (so that the DNA that has lost the stain is re-stained and is clearly visible under UV light). The stained gel is kept back in electrophoresis tank and reversed electrophoresis is carried out until the DNA ladder appears back in its sealed well. The gel slice containing DNA ladder present as a single band is removed and the gel slice is stored in buffer in refrigerator until its next use. The gel extraction using spin column can be used to purify DNA ladder from gel slice but is costlier. Therefore, the stored gel slice, when required, is placed in fresh well of agarose gel and the well is sealed with agarose. The DNA test samples that are to be analyzed are loaded in the wells of other lanes. The gel electrophoresis again resolves the DNA ladder producing bands with almost the same intensity as the fresh DNA ladder (Fig.1). The average cost of commercially available 100bp DNA ladder is Rs.40/- per gel lane. The reuse of DNA ladder helps in cutting down its cost by 50% per gel lane and costs Rs.20/- per gel lane.

It was observed that the gel slice containing the DNA ladder can be stored for a minimum of fifteen days without much loss in band intensity as seen after its re-electrophoresis.

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CONCLUSION

The reversed gel electrophoresis discussed in this paper utilizes the Tris-acetate-EDTA buffer previously used to resolve DNA ladder. The electric current polarity was reversed by interchanging the chords connecting the platinum electrodes. The gel plug containing the converged DNA ladder was removed using scalpel. Hence there is no requirement of additional reagents or time consuming steps. We conclude that the reuse of 100 bp DNA ladder is a cost-effective method that can be utilised in research laboratories.

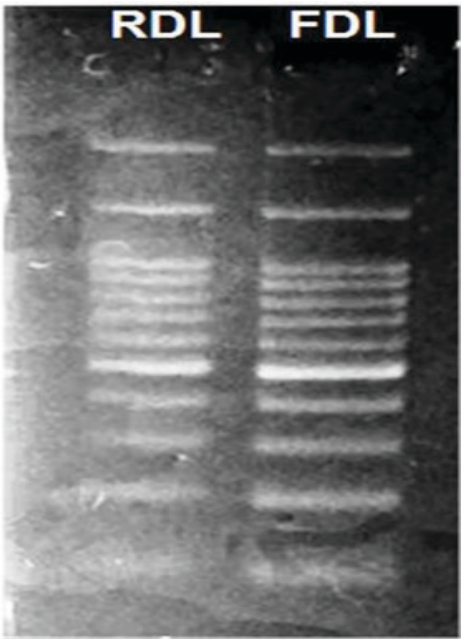

Figure 1: Reuse of 100bp DNA ladder

Gel electrophoresis was run at 100V (5.56V/cm) for 50 minutes using 3% agarose gel and 0.5X TAE. RDL, Reused DNA ladder, 100bp DNA ladder resolved again from agarose plug which was collected previously by reversed gel electrophoresis, FDL, Fresh DNA ladder, Fresh 100bp DNA ladder.

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Conflict of Interest

The authors don't have any conflict of interest.

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