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Use of Plastic Primer Container Covers as Effective Plug Molds for Pulsed-Field Gel Electrophoresis

[1]Kah Heng See, [1]Kek Heng Chua and [2]S.D. Puthucheary
[1]Department of Molecular Medicine,
[2]Department of Medical Microbiology, Faculty of Medicine, University of Malaya,
50603 Kuala Lumpur, Malaysia

Abstract: Plug molds used for PFGE can be expensive to purchase and often physically and experimentally inflexible. The use of plastic primer container covers as plug molds for Pulsed-Field Gel Electrophoresis (PFGE) is reported in this study. These plastic covers are cheap, effective and easy-to-use for processing samples for electrophoresis. Besides, the broad surface and flat area in the mold promotes faster cooling and the agar sets quickly at room temperature. Plugs are also easily removed with a spatula. These containers can be easily cleaned for re-use or disposed of as waste. Twenty samples can be prepared in one container. The PFGE results obtained by using these plastic primer-container covers as plug molds are similar to the PFGE results obtained by using commercial plug molds.

Key words: Recycle, alternative, cheap, plug mold

INTRODUCTION

Embedding bacterial cells in agarose plugs prevents damage to large DNA fragments from shear force and it is an important step in the preparation, treatment and digestion of plugs for Pulsed-Field Gel Electrophoresis (PFGE). Embedding is usually carried out using disposable or reusable commercial plug molds which can be expensive to purchase and often physically and experimentally inflexible. Removal of agarose blocks from small rigid molds can be cumbersome and challenging and difficult to clean between uses. Plug molds custom-made from silicone tubing (Burmeister and Ulanovsky, 1992), plastic syringes (Ho and Monaco, 1995), rubber dental impression material (Van Devanter *et al*., 1989), TygonR tubing, plastic drinking straw, glass pipettes (Birren and Lai, 1993), transfer pipettes (Sheneman and Katz, 2003) and acrylic molds (Herschleb *et al*., 2007) have all been reported. In this study, an initiative has been taken to transform the primer container covers obtained from First Base Malaysia into PFGE plug molds and study the PFGE patterns generated by the preparations.

MATERIALS AND METHODS

The cover of a plastic primer container, from First Base (Malaysia), was found to be suitable to be used as a plug mold for present PFGE work. This fairly soft plastic cover consists of two rows of trough-shaped hollows with numbers 1 to 5 on the top row and numbers 6 to 10 on the bottom row (Fig. 1). The original and permanent numbering on the container is very useful for labeling the plugs. Commercially purchased reusable plug molds measure 15×10×1 mm in size and are able to contain a volume of 150 µL, which gives a plug thickness of 1 mm. Therefore, to prepare an equivalent thickness in the trough of our in-house mold, which is 50×15×1 mm, we marked an area 15×15 mm to contain 225 µL of agarose cell suspension. The marking was done on the reverse side of the container for convenience and permanence. Both ends of each trough can be used to accommodate two samples at a time providing space for 20 samples in one primer container cover in the in-house plug mold (Fig. 1).

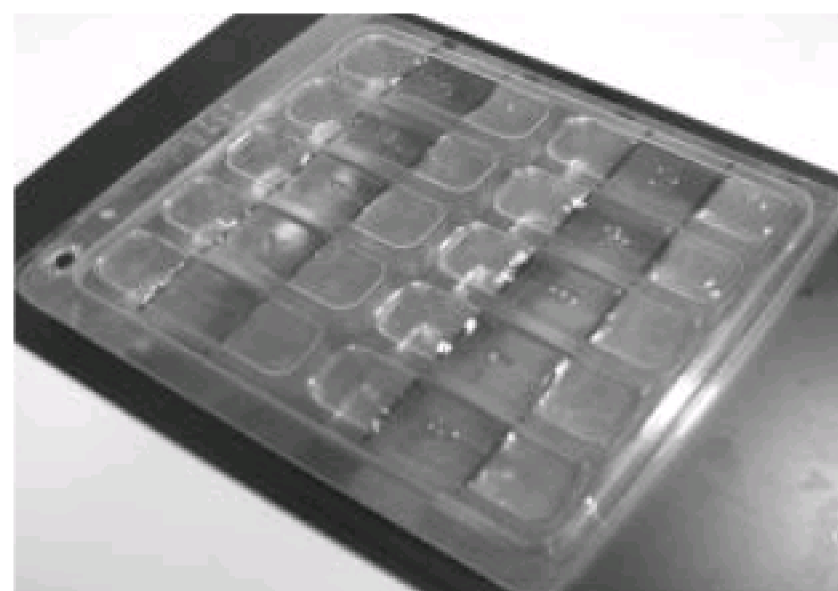

Fig. 1: Plastic primer container cover used in preparing 20 sample plugs for PFGE

Corresponding Author: Kek Heng Chua, Department of Molecular Medicine, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia Tel: +603- 79676607 Fax: +603- 79676600

DNA preparation, restriction, electrophoresis, staining and gel documentation was carried out based on the method of Ribot *et al*. (2006) with modification of the total volume of cell suspension and agarose to 225 µL including 10 µg of Proteinase K (Promega). Digestion was carried out with restriction enzyme NEB Xba1 concentration of 10 U μL^{-1} per plug and overnight incubation at 37°C. A CHEF-DR II system (Bio-Rad Laboratories, Munich, Germany) was used with the following parameters: initial switch time of 5 sec, final switch time of 65 sec, voltage of 200, angle of 120° and run time of 28 h. *Salmonella* ser. Braenderup H9812 was included as a molecular size standard. Reproducibility of the PFGE profiles was established by repeated testing of the same preparations on separate occasions.

DNA restriction patterns were analyzed for similarity by Dice Co-efficient and cluster analysis was performed by the unweighted pair group matching band average (UPGMA) of Bionumerics Version 5.1 (Applied Maths, Belgium).

RESULTS AND DISCUSSION

Present foremost objective of this study was that the PFGE patterns generated by preparations using commercial plug molds and the in-house plug molds should be identical. This was found to be true when the PFGE profiles, generated by the use of enzyme NEB Xba1, were analyzed by the Dice-coefficient of similarity, F, which expresses the proportion of shared DNA fragments of 2 preparations. In addition, the resolution and reproducibility of the band profiles were identical and comparable (Fig. 2).

The dendrogram generated by the cluster analysis of the results show a profile similarity of 100% for

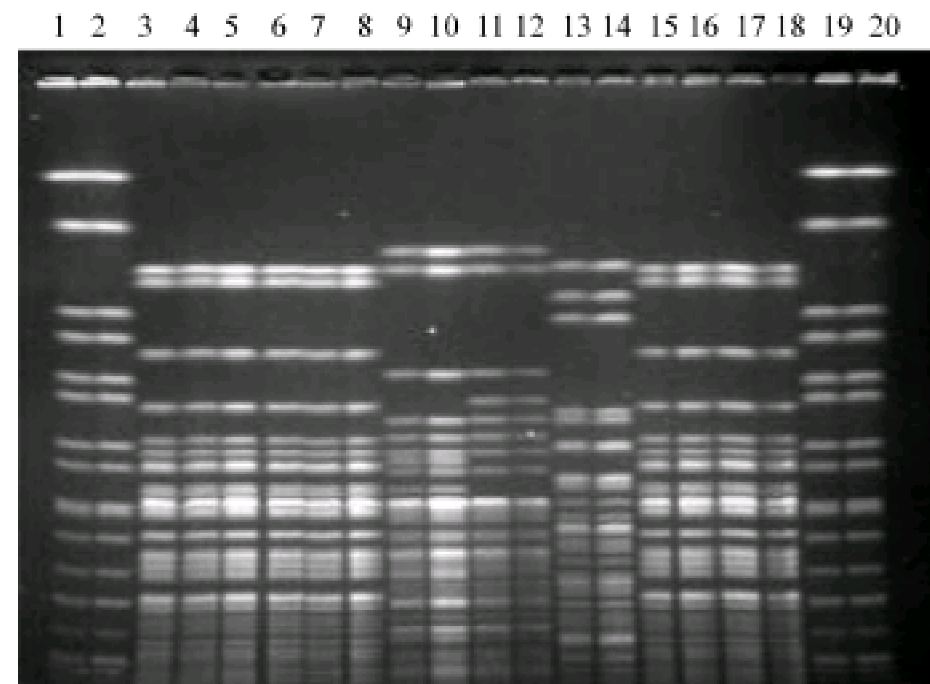

Fig. 2: PFGE profiles using restriction enzyme-NEB Xba1. Lanes 1, 2, 19 and 20 are *Salmonella* ser. Braenderup H9812 universal standard. The rest of 16 lanes– odd numbered lanes are plugs prepared in commercial molds and even numbered lanes plugs prepared in plastic primer container covers

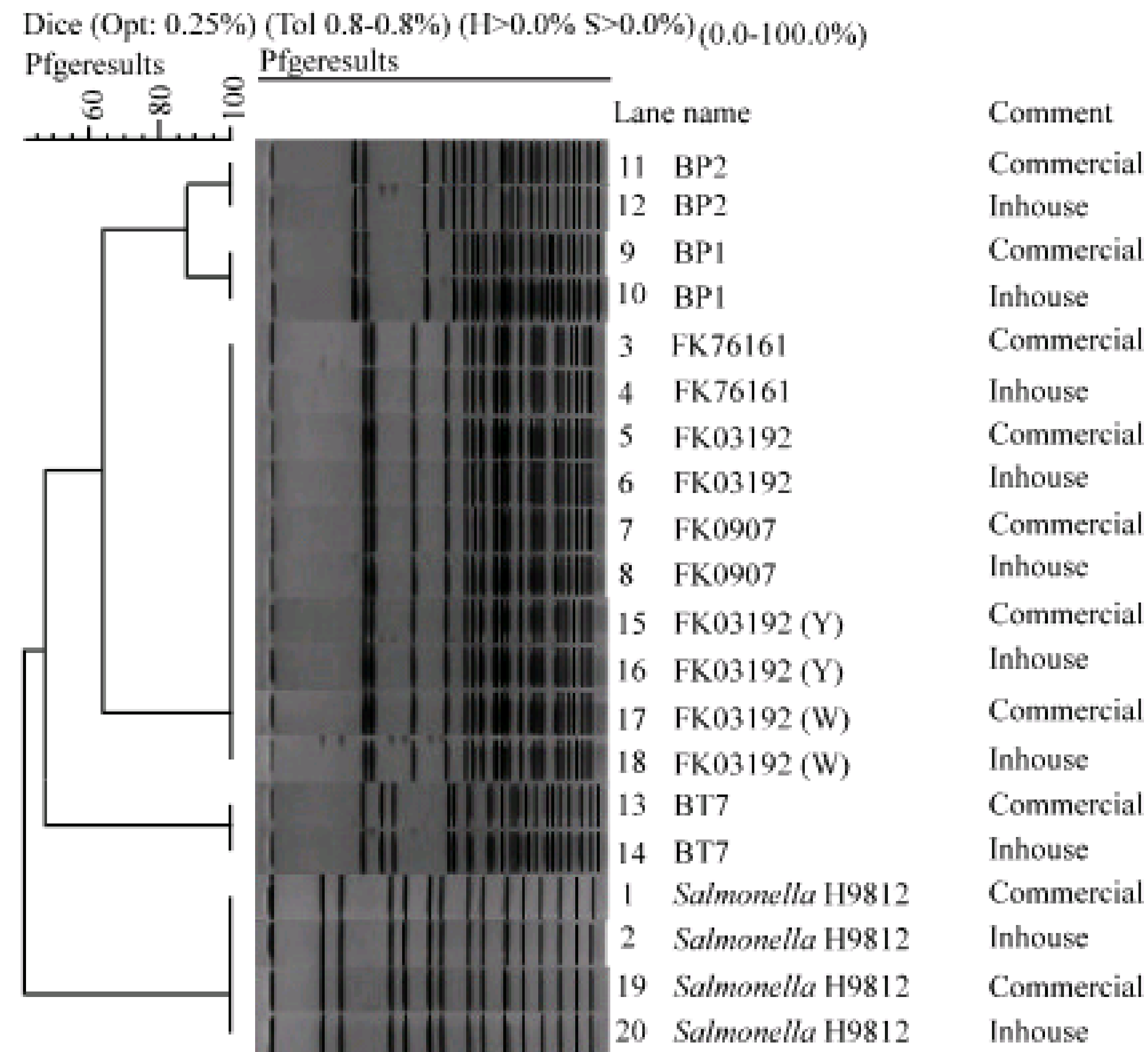

Fig. 3: Dendrogram of cluster analysis (generated by Bionumerics version 5.1 program using the UPGMA method), from plugs using commercial and primer-container molds. Identical results indicate no difference between preparations in either type of mold

Salmonella ser. Braenderup H9812, used a DNA size marker, regardless of the type of mold used. Identical results of similarity were also obtained with the other samples for both in house and commercial plug molds used (Fig. 3).

Generally, most laboratories do not purchase more than one commercial plug mold due to cost and there are limits to the number of samples that each mold can contain. With the in-house plug molds, using plastic primer container covers, 100 sample plugs can be prepared at the same time with just 5 of these covers. Added advantages are the in-house molds are cheap, easily available, recyclable and easy to clean and maintain unlike commercial molds which are usually square, enclosed and unreachable due to it small and narrow size. The broad surface and flat area in the mold promotes faster cooling and the agar sets quickly at room temperature. Plugs are easily removed using a spatula leaving no residue behind which makes cleaning easier and drying faster. It is not necessary to use tapes or parafilms and no extra labeling is required as there are permanent numbers on the covers. No clamping or cutting of the mold is needed to remove the plugs.

Overall, the easy to-use molds provide flexibility for producing different sizes and thickness of plugs by controlling the volume of cells and agar to be dispensed into the molds. They are also more user friendly compared to other custom made, usually rigid plug molds with other materials, as reported by Burmeister and Ulanovsky (1992), Ho and Monaco (1995), Van Devanter *et al*. (1989), Birren and Lai (1993) and Sheneman and Katz (2003).

CONCLUSION

Plastic primer container covers can be recycled and used as plug molds for pulsed-field electrophoresis. Researches who are working on PFGE can use this alternative method instead of using the expensive commercial plug molds.

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