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Note

Simple gel apparatus for horizontal polyacrylamide and agarose gel electrophoresis

ULRICH CERTA* and DETLEF WESSEL
Department of Molecular Biology, Max-Planck-Institute for Experimental Medicine, 3400 Göttingen (G.F.R.)
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Vertical gel systems are widely used in agarose and polyacrylamide gel electrophoresis. Air bubbles generated while pouring the gel and leakiness of the buffer chambers are problems that often occur. Restriction analysis of DNA fragments[1] and the Southern blotting technique[2] require good separations and sharp bands. In vertical gel systems the bands usually are not sharp owing to diffusion of the samples in the direction of separation. This can be overcome by using horizontal slab gels. As polyacrylamide does not polymerize when exposed to air, no system was previously available for pouring horizontal polyacrylamide slab gels. We describe here a simple apparatus and demonstrate its use in separating DNA restriction fragments.

EXPERIMENTAL

Chemicals

The agarose used was Type ME from Seakem (Marine Colloids Div., FMC, Marcus Hooke, PA, U.S.A.). Acrylamide (recrystallized twice, p.a. grade) was purchased from Biomol (Ilvesheim, G.F.R.), and N,N′-methylenbisacrylamide from Serva (Heidelberg, G.F.R.). DNA standards II and III are restriction enzyme digests of bacteriophage λ DNA with ECO RI and HIND III and were obtained from Boehringer (Mannheim, G.F.R.). Other chemicals were of analytical-reagent grade.

Apparatus

All parts were assembled from commercial plexiglass in the institute's workshop. A scheme of the gel holder is shown in Fig. 1.

For pouring gels the seals (A) and the edges of the cover (C) are greased with Vaseline and connected as indicated in Fig. 1. The gel is poured through the slit (D) in the cover (C) using a disposable 50-ml syringe. The sample comb (E) is inserted into the slit (D). After polymerization the seals (A) and the sample comb (E) are removed carefully and the apparatus is placed in the buffer chamber, which is then filled to the cover (C) with Tris–borate buffer (89 m*M* Tris, 8.9 m*M* boric acid, 2.5 m*M* EDTA, pH 8.0). Samples are dissolved in 15 μl of 25 m*M* EDTA (pH 8.0) containing 0.05% of bromphenol blue and 0.2% of agarose and heated for 10 min at 56°C before loading.

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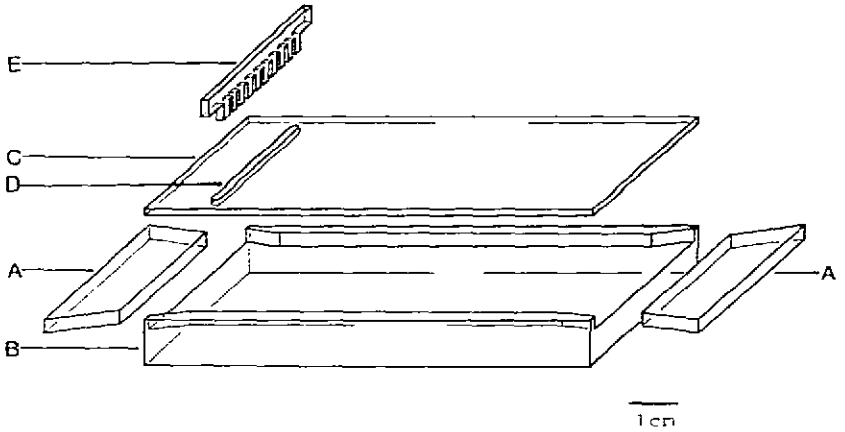

Fig. 1. Design of the gel holder. The parts are assembled as indicated. Prior to assembly the seals (A) and the edges of the cover (C) are greased with Vaseline. The gel is inserted through slit (D) using a disposable syringe. The sample comb (E) is inserted through slit (D). After polymerization of the gel, the comb and the seals (A) are removed carefully. The gels are 9 cm long and 2 or 4 mm thick, depending on the gel holder (B). In 2-mm gels the sample slots are 1 mm deep and in 4-mm gels 2 mm deep. For electrophoresis the gel holder (B) connected with the cover (C) is placed in a buffer chamber (see Fig. 2). Buffer is added up to the cover. After electrophoresis the cover is removed and the gel is stained in the gel holder to prevent damage to the gel.

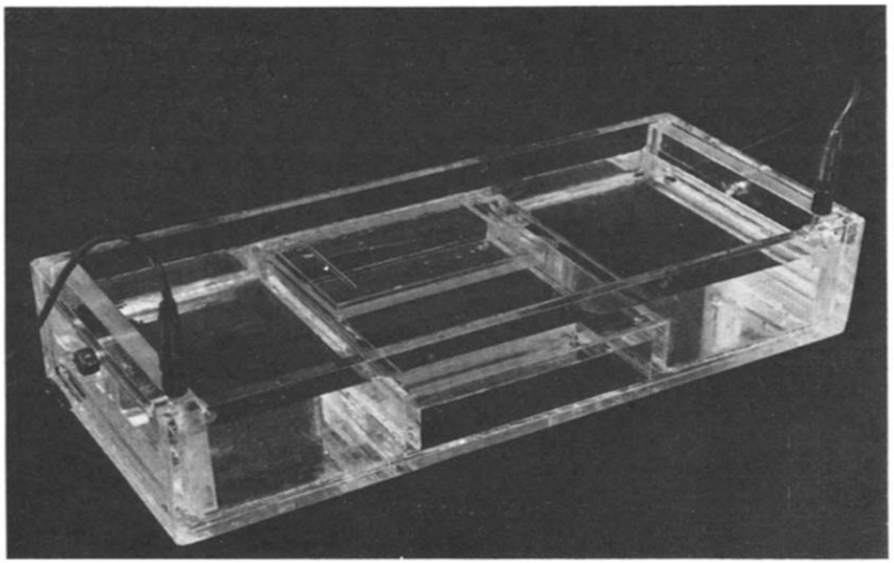

Fig. 2. Photograph of the gel system during a run. The gel holder is placed in a 30-cm long buffer chamber and filled with buffer so that the gel makes contact with the buffer. Even when the gel holder is completely overloaded with buffer good separations are obtained. Direction of separation is from left (−) to right (+). The buffer chamber is not described in detail as other similar chambers can be used. During electrophoresis the buffer chamber is closed with a cover to prevent evaporation of buffer (not shown).

Agarose gels

Agarose was dissolved in Tris–borate buffer (see above) and heated for 3 min using a microwave oven (Philips Model 810 D). After cooling to 70 C the gel was

poured as described above. After cooling to room temperature the samples were loaded and electrophoresis was run at a constant voltage of 1 V/cm for 12 h at room temperature.

Polyacrylamide gels

A 50-ml volume of 5% acrylamide and 0.17% bisacrylamide in Tris–borate buffer was filter-sterilized and degassed for 15 min using water suction, then 50 μl of N,N,N′,N-tetramethylenediamine (TEMED) and 500 μl of 10% ammonium peroxidisulphate in water were added. The gel was poured as described above. After polymerization, the samples were loaded and electrophoresis was run at a constant current of 30 mA for 4 h at room temperature.

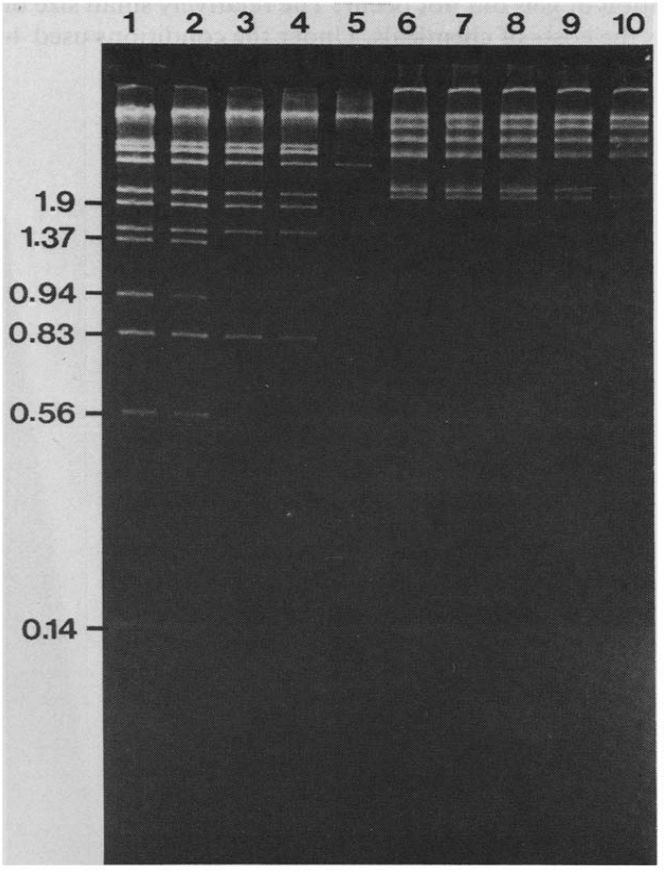

Fig. 3. Separation of DNA restriction fragments of bacteriophage λ DNA on 5% polyacrylamide gel. In lanes 1–5 were loaded 0.5, 1.0, 1.5, 2.0 and 2.5 μg of DNA digested with the restriction enzymes ECO RI and HIND III as described under Experimental. In lanes 6–10 were loaded 0.5, 1.0, 1.5, 2.0 and 2.5 μg of DNA digested with HIND III only. Electrophoresis was performed at 30 mA for 4 h. After the run the gel, in the gel holder, was stained with ethidium bromide solution (4 μg/ml) for 30 min. The bands were revealed under short-wave UV light and photographed with a Polaroid camera. The size of the fragments was given by the supplier of the standards and is indicated adjacent to the bands (kilobases). The gel was 4 mm thick and 9 cm long. Direction of electrophoresis is from top (−) to bottom (+).

Staining

The gels were stained for 30 min in 4 μg/ml ethidium bromide solution and the bands were revealed by illumination with short-wave UV light.

Photography

The gels were photographed with a Polaroid camera using Type 55 film and an orange filter to reduce background.

RESULTS AND DISCUSSION

The apparatus was applied to the separation of DNA restriction fragments. As shown in Figs. 3 and 4, the bands are much sharper than those on conventional gel slabs. Problems with polymerization of gels did not occur. The relatively small size of the gel layer (7 × 10 cm) reduces the costs of chemicals. Under the conditions used 4-

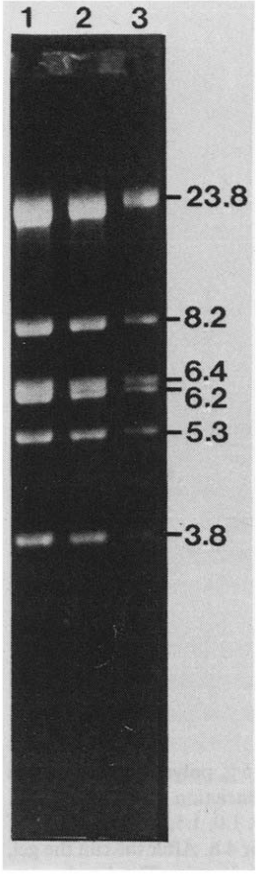

Fig. 4. Separation of DNA restriction fragments of bacteriophage λ DNA on a 0.7% agarose gel. In lanes 1–3 were loaded 1.0, 0.6 and 0.3 μg of DNA digested with the restriction enzyme ECO RI. Electrophoresis was carried out at 1 V/cm for 12 h. Other details as in Fig. 3.

mm thick gels gave the best results for polyacrylamide and 2-mm thick gels gave the best results for agarose.

Southern blots[2] routinely carried out gave excellent results (data not shown). The simplicity and convenient handling of the apparatus makes it applicable to most gel techniques, including discontinuous electrophoresis. Gels can be stained when still in the gel holder, which is advantageous when using low percentage gels or radioactively labelled samples.

As the apparatus is not available commercially, detailed construction plans can be obtained from the authors on request.

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