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Simultaneous loading of 200 sample lanes for DNA sequencing on vertical and horizontal, standard and ultrathin gels

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ABSTRACT

We have developed a simple and efficient technique for automated parallel loading of ≥200 lanes on a 30 cm-wide gel in automated DNA sequencing, using porous filter materials and an associated manual or robotic system. The samples are loaded onto the teeth of a comb made of the porous material. The comb, with samples, is inserted directly above the straight edge of the polymerized gel. The samples are driven from the comb into the gel by the applied electrical field. A particularly advantageous aspect of this method is the elimination of the thin gel walls separating the sample wells in the standard gel loading technique. The time for sample loading is significantly reduced to a few minutes. The loading technique is applicable to horizontal or vertical systems, with standard or ultrathin gels.

In the standard gel casting and loading protocols, a plastic comb is used to form sample wells in the polymerized gel. The plastic comb is removed after polymerization, samples loaded in the wells, electric field applied and the separation process started. Problems with this technique are encountered when dimensions of the sample slots are small, i.e., for widths <2 mm. In systems with high throughput, large numbers of samples per gel are required, and the slot width may be ≤1.5 mm. When the gel walls separating the slots are very thin (<1 mm) they may become damaged, leading to mixing of different samples, distorted band patterns and unreliable results. The problem is worse with gels <0.5 mm thick. The 'shark tooth' technique avoids the problem of thin separating walls, but the manual sequential loading into the small sample slots remains tedious, time consuming and prone to errors.

We have developed a simple and efficient technique for automated parallel loading of ≥200 lanes on gel in automated DNA sequencing, using combs made of porous filter materials and an associated manual or robotic system. The gel used was 5% Hydrolink, 1× TBE. Casting of the gel and sequencing protocols were as described in ref. (1). To prepare the straight and smooth edge of the gel on which the samples are loaded, a polyester spacer with a straight and smooth edge, of the thickness of the gel, is inserted ~5 mm inside between the glass plates, similarly to the technique for DNA sequencing on ultrathin gels described previously (2), and the gel solution is added. After the gel polymerises, the polyester spacer is removed leaving the gel side with a straight boundary. The sequencing runs were performed on an ALF or EMBL 2-dye system (3) with array detectors.

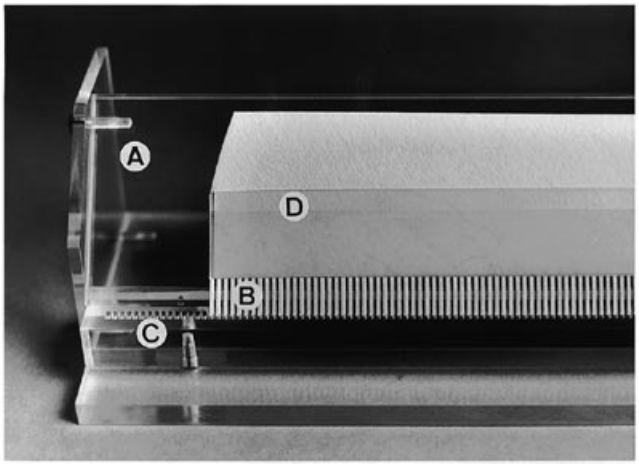

Figure 1. Sample block (A), with the teeth of the porous comb (B) inserted in the sample pockets (C) for drawing the samples in the comb. Optionally, a plastic strip (D; polyester, width 20 mm, thickness 0.3 mm) is fixed to the comb above the teeth as indicated, to improve its handling.

The comb is cut out of the porous material in a mechanical workshop using a standard circular saw-blade (0.6 mm thick). The dimensions of the teeth are 1 mm wide, 8 mm long and the distance between them is 0.6 mm. The porous material used for production of the loading comb was MV Cellulose–Mischester from Macherey-Nagel, Düren or Nylon Membrane from Boehringer Mannheim. Optionally, a plastic strip may be fixed on the comb to strengthen it and improve its handling during the sample application and its insertion between the glass plates. Sample pockets are machined out into the surface of a polyacryl glass block, at the same distance as that between the teeth of the porous comb. The application of the samples is performed in parallel by

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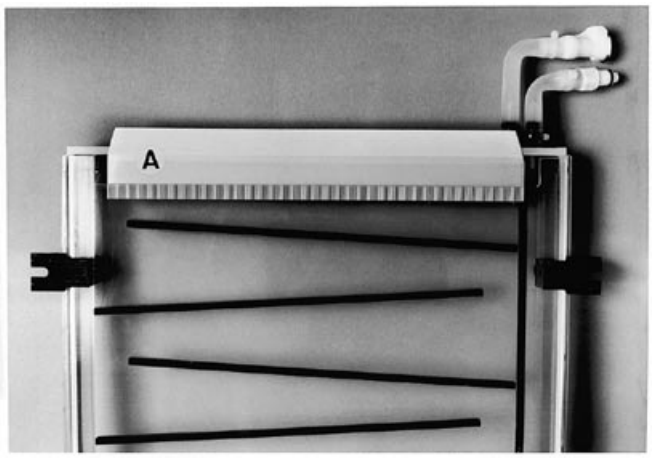

Figure 2. Gel sandwich with porous comb (A) inserted.

lowering the comb teeth into pockets containing the sample liquid (Fig. 1). The samples are drawn by capillarity into the teeth of the porous comb. The liquid sample volumes (1–2 µl) were filled previously in the sample pockets in the block out of microtiter plates, manually, or by a robotic dispense system. The comb containing the samples was inserted between the glass plates above the polymerized gel (Fig. 2) usually within 20 min after its loading. After insertion of the sample comb, electrode buffer is added, the electric field switched on and sequencing run started.

The technique has worked routinely in our laboratory for 1 year for 40–200 lanes on one gel. Single dye readings ~1000 bases, and with the two-dye EMBL system 2000 bases per sequence reaction, were obtained on 60 cm-long glass plates with very high accuracy (Fig. 3). The teeth of the comb are either in direct contact with the gel, or are placed at some distance (several millimetres) above the gel upper smooth boundary. In several tests we have inserted the loaded sample comb between the glass plates prior to addition of the gel solution, and the sample comb was polymerized in the gel. Readings ~1000 bases were obtained, but the single peak resolution seems slightly inferior to the technique described above.

The technique allows sequential sample comb applications to the same gel; it may be useful for quick diagnostic screening or mixing several samples prior to loading, adjustment of final sample volumes to 1–2 µl and simultaneous application on one comb to the gel. It can be applied with all standard buffers and the usual fluorescent as well as radioactive labels, with DNA or protein samples. The loading technique was tested with polyacrylamide and agarose gels in horizontal and vertical systems, and it should be also applicable to uncrosslinked polymer networks, e.g. linear polyacrylamide, hydroxyethyl–cellulose and other novel separation media.

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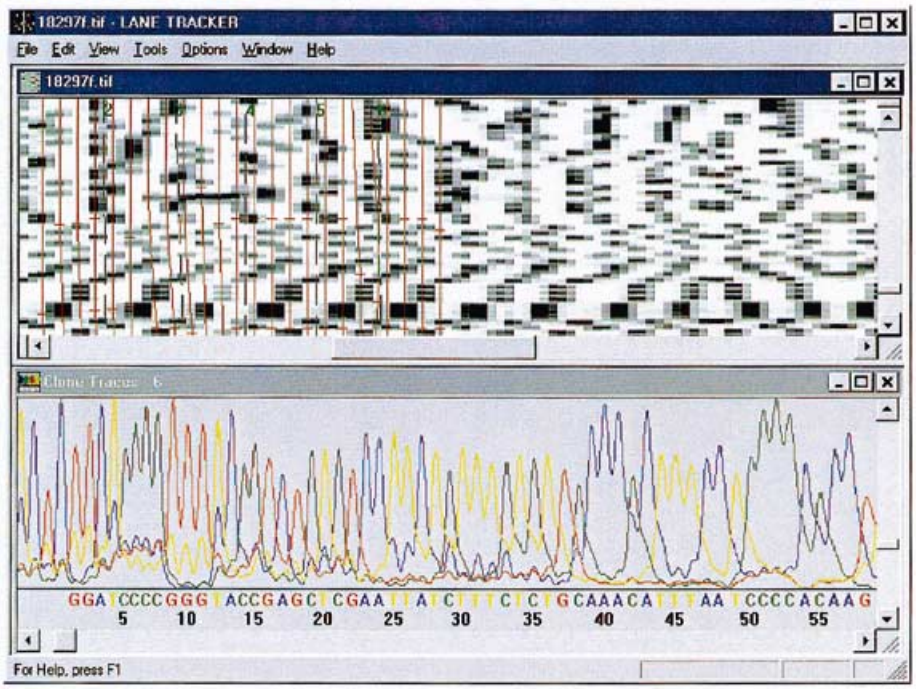

Figure 3. Computer display of sequence data obtained with the filter loading technique. Sample teeth width 1 mm, EMBL 2-dye array sequencer for 80 clones, 5% Hydrolink gel, thickness 0.35 mm.