Pulsed field gel electrophoresis using a double-decker gel system

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Pulsed field gel electrophoresis (PFGE) is capable of resolving large DNA molecules of up to two million nucleotides and is becoming a widely used technique in DNA studies (1–3). Three factors can potentially limit PFGE in a laboratory: 1. the cost of the apparatus, 2. reproducibility of electrophoretic conditions, and 3. unusually long electrophoretic time. To alleviate some of these problems we have tested the simultaneous electrophoresis of two gels in a double-decker setup using a Bio Rad Chef-Dr II apparatus. By employing a buffer flow rate of 0.5 l/min the upper gel is stabilized in a completely flat and horizontal position supported on top of the slightly protruding corners and edges of the bottom gel (Fig. 1). Subsequent Southern hybridization showed a complete lack of cross contamination between the two gels (Fig. 2). Under the electrophoretic conditions used in Fig. 2, running double-decker gels resulted in a 6% reduction in the distance travelled by the DNA in comparison to the use of a single gel. We have noted that the two gels in the double-decker gave identical DNA resolution (Fig. 2), making it feasible to perform accurate linkage mapping of adjacent genetic loci by overlaying the two resulting Southern autoradiograms and searching for common bands (unpublished results). This contrasts with a 5% difference between gels electrophoresed under the same conditions in two separate sets of Chef-Dr II apparatus. Placing a dialysis membrane (not routinely used by us) between the gels had no effect, whereas a sheet of plastic (overhead transparency) deformed the gel tracks in the upper gel significantly (data not shown). The described double-decker procedure therefore offers the advantage of doubling our PFGE capacity, and provides us with gels that can be directly compared to each other.

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REFERENCES

Figure 2. Southern blot analysis of the upper (A) and lower (B) gels resolved using the double-decker system. A total of 11 *Saccharomyces cerevisiae* chromosome agarose blocks were loaded into the two gels. The 6 blocks in the upper (A) gel and the 5 blocks in the lower (B) gel were positioned to avoid overlapping tracks in the two gels, so that any transfer between gels would be seen. I, ethidium bromide staining of the gels; II, Southern hybridization using 32P-labelled yeast DNA as a probe. Note the absence of any cross-contamination of DNA between the two gels even after prolonged autoradiographic exposure. PFGE conditions were: 60 second pulse time for 15 hours followed by a 90 second pulse time for 9 hours at 200 volts, using a 0.8% agarose gel in 3 liters of 0.5 X TBE buffer circulating at a rate of 0.5 l/min at 10°C.