In-gel hybridization of DNA separated by pulsed field agarose gel electrophoresis

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Methods for the in-gel hybridization of DNA overcome variability of hybridization caused by the transfer usually performed before hybridization (1, 2). However, previous procedures of in-gel hybridization have been limited by: (a) removal from the gel of liquid phase water (and, therefore, possibly DNA) during drying, (b) gel curling during drying, and (c) gel disintegration during washing at either 65°C or higher. The following procedure for in-gel hybridization overcomes these limitations and has been used after field inversion gel electrophoresis (FIGE), known (3) to cause greater-than-usual problems during transfer. After FIGE of bacteriophage T7 concatemers produced (4) at 0°C (3 V/cm, ramped 9–30 sec forward, 3–10 sec reverse; 15°C, 43 hr), a 1.5% Seakem LE agarose gel in 0.01M sodium phosphate, pH 7.4, 0.001 M EDTA was washed for 1–2 hr in distilled water and then air-dried on a sheet of plastic at 56°C, for 10–16 hr. DNA in the gel was rehydrated, denatured, neutralized, rinsed 3–4 times with distilled water and hybridized (3) to T7 DNA ³²P-labeled by use of nick translation. After washing (3) for 2 hr at 55°C, glycerol was added to 12% and the gel was air-dried a second time at 42°C. The dried gel was washed (3) at 65°C (70°C is the limit), brought to 12% glycerol and then air-dried a third time. The result is a flat gel with sharp bands of concatemers; between bands is in-lane background from concatemers variable in length (Fig. 1). Cross-hybridization with non-homologous DNA was not observed. Sensitivity and background are comparable to those of our best Southern blots; reproducibility is greater. Smooth, monotonic response to DNA amount (0.001–1.0 ng/band) was observed. The glycerol prevented both gel curling during drying and gel disruption during washing. Glycerol was not used during the first drying because it caused an increase in background. A second denaturation did not remove the probe.

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Figure 1. T7 Concatemers. (a) 3.6 ng; (b) 1.2 ng total. Arrows: Length in 40 Kb units.

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