Blunt-ended ligation can be used to produce DNA ladders with rung spacing as large as 0.17 Mb

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The blunt-ended ligation of 0.04 Mb and longer DNA is needed for both insert-vector joining and the production of DNA ladders used as length standards during pulsed-field gel electrophoresis. Blunt-ended ligation was achieved for mature bacteriophage T4 DNA (0.17 Mb) by adding 3 μ l of 250 μ g/ml DNA to a mixture consisting of: (a) 27 μ l of 14% (w/w) polyethylene glycol 6000 (PEG) in 0.1 M sodium chloride, 0.01 M sodium phosphate, pH 7.4, 0.001 M EDTA, (b) 8 μ l of 0.25 M Tris-Cl, pH 7.4, 0.05 M MgCl₂, 0.1 M dithiothreitol, 0.005 M ATP, 250 μ g/ml bovine serum albumin, and (c) 1 μ l of 3.3 units/ μ l T4 ligase (U.S. Biochemicals). After incubation at 37°C for 6 hr and inactivation of the ligase at 75°C for 15 min, a DNA ladder with 0.17 Mb rungs was observed by use of rotating gel electrophoresis (RGE) (1) for 36 hr, at 2 V/cm, 20°C in a 1% Seakem LE agarose gel cast in 0.01 M sodium phosphate, pH 7.4, 0.001 M EDTA; the pulse time was 120 sec and the angle of rotation was 1.4π radians (Fig. 1a, lane 1). The T4 ladder was not formed when the PEG was omitted (Fig. 1a, lane 2). When the ligation of Fig. 1a, lane 1 was performed by use of 0.04 Mb T7 DNA, a 11–12 rung ladder was observed (Fig. 1b). Restriction endonuclease analysis of the T7 ladder revealed the expected left-left, left-right and right-right restriction endonuclease fragments (not shown). Thus far, the length of the T4 ladder appears limited by the amount of broken DNA in the ligation mixture. The stimulation by PEG of the blunt-ended ligation of shorter DNA has previously (2) been observed.

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REFERENCES

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Figure 1. (a) T4; (b) T7 ladders. The gels were stained with ethidium. The length of DNA in Mb is indicated.

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