Rapid method for preparation and cleavage of bacterial DNA for pulsed-field gel electrophoresis

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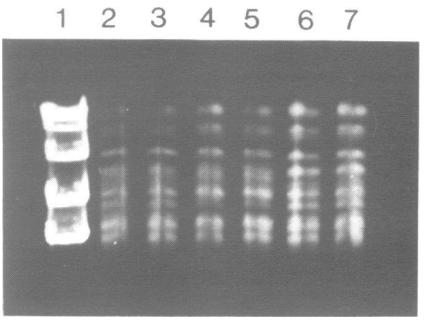
The following very simple "mini-prep" procedure has been routinely used in our laboratory for over 100 extractions from *Bacillus subtilis* and from *Escherichia coli,* permitting the time between harvesting of bacterial cells and loading of DNA onto a pulsed-field gel to be reduced to less than five hours. It took advantage of the fact that chromosomal DNA is more loosely associated with histone-like proteins in bacteria than in eukaryotes (1). The method was based in part upon osmolysis in the presence of ethylene glycol-bis(ß-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA) to inhibit certain nucleases (2). Bacteria (1 to 2.5 ml) were grown in a gyrotary waterbath at 37°C to either mid or late exponential phase in rich media. Pellets were suspended in 1 ml buffer A (20 mM Tris·HCl, pH 8, 20 mM $MgSO_4$, 20% sucrose, 1 mM EGTA), then resuspended in 250 µl buffer A. *B. subtilis* was then processed immediately, whereas *E. coli* was subjected to a rapid freeze-thaw before further analysis. Lysozyme (0.4 mg/ml final) was then added and incubation performed with gyrotary shaking at 37°C for about 30 min, and spheroplast formation was confirmed by phase-contrast microscopy. It was important that cells were kept shaking at this stage. An equal volume of 1% (w/v) low-melting-temperature agarose (SeaPlaque, FMC Bioproducts) in buffer A at 37°C was added with swirling. Aliquots (20 µl) were dispensed onto a sheet of Parafilm resting on a flat bed of ice and allowed to solidify (15 min). For each digest, two plugs were placed in ice-cold 150 µl buffer B (20 mM Tris·HCl, pH 7, 5 mM $MgSO_4$, 50 mM NaCl, 1 mM EGTA, 1 mM dithiothreitol) and left at 0°C for 1 hr. After aspiration, ice-cold 150 µl restriction enzyme buffer (made up from stock supplied by Promega Biotech) was added, and left for 15 min at 0°C, then repeated. *Not* I (typically 16-40 U; Promega Biotech) was added; reactions were usually for 2 hr, although complete digests have been obtained in as little as 1 hr with more enzyme, as judged by Southern analysis. The small size of the agarose plugs facilitated rapid diffusion of restriction enzymes and subsequent digestion. Plugs (2 per lane) were usually pushed into the well by means of a spatula and bent glass rod, followed by contour-clamped homogeneous electric field (CHEF) electrophoresis (3) and Southern analysis. Figure shows lambda multimers (lane 1), *B. subtilis* KS306 (lanes 2,3), BZ213 (lanes 4,5) and KS297 (lanes 6,7), with little background smear. The DNA produced using the procedure described here has also been successfully used for additional enzyme reactions, e.g. labelling with polynucleotide kinase and Klenow followed by several changes of Tris·EDTA buffer prior to electrophoresis. This work was supported by a URG Special Projects Grant and by the University of Sydney Cancer Research Fund.



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