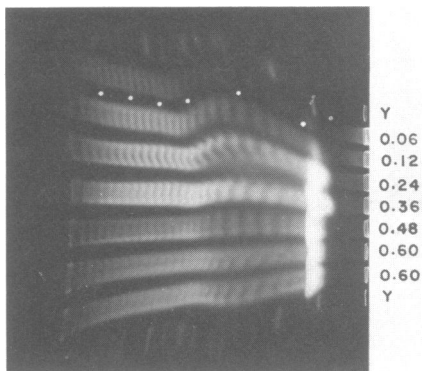

Generation of lambda phage concatemers for use as pulsed field electrophoresis size markers

P.Greg Waterbury and Michael J.Lane

Department of Medicine, Division of Hematology/Oncology, State University of New York, Health Science Center at Syracuse, Syracuse, NY 13210, USA
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Pulsed field electrophoresis (1,2) makes possible the separation and resolution of DNA molecules up to millions of base pairs in length. Here we describe a simple, rapid, and reproducible method for generating concatemers of lambda phage to be used as molecular weight markers in such experiments. Wild type whole lambda phage (Ci857sam7), purified over a cesium chloride gradient, were purchased from StrataGene Cloning Systems, San Diego, CA. The phage were dialyzed overnight at 4 C in 10 mM NaCl/10 mM MgCl₂/50 mM Tris Cl (pH 8.0), and subsequently diluted in phosphate buffered saline (PBS) and mixed with an equal volume of 1.5% low gelling agarose (FMC lot #12276) making final DNA concentrations of 0.06 - 0.60 ug/ul (E(P) = 6600). The molten agarose solution was mixed and then pipetted into plastic forms and allowed to solidify making agarose 'plugs' (2). Agarose plugs were suspended in a solution composed of 0.5 M disodium EDTA/1% sarcosine/1 mg/ml proteinase K/ 10 mM Tris-Cl (pH 9.0) and incubated at least 4 hours at 55 C with gentle shaking. (Plugs may be used the same day or stored at 4 C in lysis buffer, until needed.) Samples were loaded as described (2) onto a 1% agarose gel for electrophoresis. The gel shown was run for 62 hours at 8.5 V/cm with a pulse time of 150 seconds on an apparatus made in this laboratory. Gel lanes are labelled by concentration (ug/ul) except for the two outside lanes which show chromosomes prepared from the yeast strain DBY 745 for reference. DNA sizes are indicated by dots every 200,000 bp in the 0.12 ug/ul lane. As can be seen the degree of concatemerization is greater as the concentration of phage DNA increases. The optimal concentration of phage DNA necessary for obtaining good 'ladders' is between 0.12 and 0.36 ug/ul.



- Ref** 1. Schwartz, D.C. and Cantor, C.R. (1984) *Cell* **37** 67-75.
2. Schwartz, D.C. et al., (1982) *CSHSQB* **48** 189-195.