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Ramped field inversion gel electrophoresis: a cautionary note

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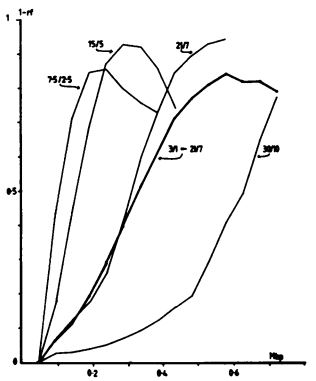

We have been using Field Inversion Gel Electrophoresis [1] to study the organization of plant genomes. A major difficulty with this approach is that DNA molecules do not migrate in sequential order of size, but it has been suggested that a steady change of pulse length during a run (pulse ramping) can overcome the problem [1,2]; our results do not completely support this contention. We have investigated this phenomenon using two dimensional gel runs to resolve λ oligomers prepared according to the method of Anand [2]. The first and second dimensions differ in the pulse regime employed, allowing us to determine, from the order of bands in both dimensions, the mobility of defined λ oligomers. 1.5% agarose gels were run in Tris-acetate buffer in a vertical gel box essentially as described by Southern [3]; the temperature was controlled by circulating water from a cooled external water-bath, through a coil immersed in the back tank. The power supply polarity, voltage, pulse and run duration was controlled by a ZX 81 microcomputer; the power supply and interface were constructed here. Run conditions used were 30 min, 100V run in, followed by 200V pulses (11V cm^{-1}). Runs were compared by measuring the mobility of λ oligomers and expressing this as a fraction (rf) of the mobility of the λ monomer. The graph shows plots of (1-rf) vs size in Mbp. The pulse regime is indicated as x/y where x is the forward pulse time and y is the back pulse time (sec). For the ramped run (bold line) starting pulses were x=3, y=1 and steadily increased for 16hrs to x=21, y=7. The plots show that the ramped run extends the range of oligomers separated in sequential order of size, but with the largest oligomers still out of sequence, so ramping does not entirely overcome this problem. The shallow slope of the plot for ramped runs implies that the resolution of the gel at a given size is reduced.

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3 Southern EM (1979) Method. in Enzymol. 68 152-176