Resolution of DNA fragments from 23 kilobases to 6 megabases by biphasic linear pulse ramping

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DNA fragments under 200 kilobase pairs (kbp), between 200 kbp and 2 megabase pairs (Mbp), and between 2 Mbp and 6 Mbp are optimally separated by pulsed field gel electrophoretic techniques using different switching frequencies, electrophoretic field strengths, angles of reorientation, and/or agarose concentrations (1). For this reason, analyses of uncharacterized DNA fragments using pulsed-field techniques often require preliminary studies to determine what size ranges DNA fragments of interest fall within and it is not uncommon to run at least two different pulsed-field gels in order to fully characterize large DNA fragments from a single cell source (e.g. 2).

A significant expenditure of time and reagents can result from the need to run multiple gels on a single set of DNA samples. It would therefore be desirable to identify a relatively simple strategy for linear resolution of DNA fragments from under 200 kbp to 6 Mbp in a single pulsed-field gel. Since most commercially available pulsed-field systems are not capable of changing applied field strengths within a run (1), and many have fixed angles of reorientation (1), a separation strategy relying only upon manipulation of switching frequencies would be optimal.

A biphasic linear pulse ramping strategy coupled with a fixed electric field strength and angle of reorientation can provide reasonable resolution of DNA fragments from 23 kbp to 5.7 Mbp in size (Figure 1). The gel shown in Figure 1A was run on a BioRad CHEF DR-II system, which has homogeneous electric fields and a reorientation angle of 120° (1). The gel was 0.5% agarose (BioRad chromosomal grade) made in 45 mM Tris borate, 1 mM EDTA, pH 8.0 (0.5×TBE), with 0.5×TBE running buffer recirculated at 10°C. The applied electric field was held constant at 1.95 V/cm (65 V), and pulses were ramped linearly from 30 seconds to 2 minutes over 27 hrs and then from 2 minutes to 50 minutes over 46 hrs. Under these conditions, the chromosomes of *S.pombe* (BioRad), *S.cerevisiae* (BioRad), lambda phage concatomers (FMC), and the larger fragments of lambda phage digested with HindIII (Boehringer Mannheim) migrate as an approximate linear function of the log of their respective molecular weights (in Mbp, Figure 1B). Nine chromosomes of *S.cerevisiae* are between 580 kbp and 1.1 Mbp in size, making identification of individual chromosome bands difficult in this region. Bracketted points in Figure 1B represent estimations of the migrations of the two chromosomes on either end of this region (580 kbp and 1.1 Mbp).

The strategy outlined above is not ideal for sharp resolution of bands within a particular narrow range of DNA fragment sizes, but can be extremely useful for determining what particular

fragment size ranges deserve closer examination. For this reason, it is a preferable approach to running multiple pulsed-field gels under different conditions when size ranges of greatest interest remain unknown.

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Figure 1. Resolution of DNA fragments ranging from 23 kbp to 5.7 mbp in size by biphasic linear pulse ramping. **A.** Ethidium bromide stain of chromosomes of *S.pombe* (lane 1) and *S.cerevisiae* (lane 2), lambda concatomers (lane 3), and 27.5 kbp and 23.1 kbp lambda HindIII fragments (lane 4). Migration points of the four smallest lambda concatomers (48.5, 97, 145.5, and 194 kbp in size) are noted with large arrowheads on the left, while migration points of the 23.1 and 27.5 kbp bands of lambda/HindIII digest are noted with small arrowheads. Specific electrophoretic conditions are described in the text. **B.** Semi-log plot of the sizes of individual DNA fragments (in Mbp) versus distance migrated from the origin (in cm). Bracketted points are extrapolated from (A) (see text).