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Resolution of *Schizosaccharomyces pombe* chromosomes by field inversion gel electrophoresis

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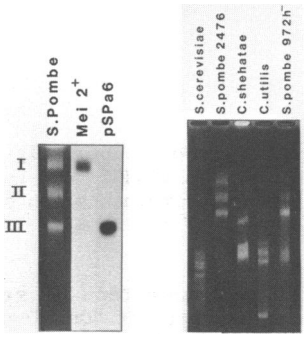
The upper size range of electrophoretic separation has recently been extended to more than 5,000 kbp (1). Here we describe the use of field inversion gel electrophoresis (FIGE) (2) to separate S. pombe chromosomes.

Yeast DNA samples were prepared in plugs of 0.5% agarose and subjected to FIGE as described previously (3) using an intervalometer (Sound Scientific, Seattle, WA) and two 250V power supplies to apply a periodic sequence of field pulses such that a "forward" (direction of migration) polarity electric field, E_+, of 1.3 V/cm was first applied for a fixed period of time, T_+, followed by a second field of "inverse" polarity, E_-, of -0.5 V/cm for a fixed period of time, T_-. In figure 1, the three chromosomes of S. pombe (ATCC strain 2476) are clearly resolved using the following pulse sequence: T_+=30s and T_-=30s during the first 16h of electrophoresis and T_+=3000s, T_-=1200s during the next 80h of electrophoresis. The identity of the electrophoretic bands have been verified by Southern transfer and hybridization using the Mei2$^+$ (provided by D. Beach, Cold Spring Harbor, NY) and pSPa6 (provided by S.A. Nadin-Davis, NRC Canada, Ottawa, Ont.) DNA probes which are specific for chromosomes I and III, respectively. In figure 2, the chromosomes of S. cerevisiae (strain A364a) are clearly resolved as are those of the two S. pombe strains ATCC 2476 and ATCC 24843 (972 h$^-$) as well as those of C. shehatae and C. utilis by using the following pulse sequence: T_+=3000s, T_-=1200s during the first 80h of electrophoresis; T_+=1500s, T_-=600s during the second 42h of FIGE and T_+=300s, T_-=120s during the last 45h of the experiment. These results indicate that very large DNA fragments can be distinctly separated (figure 1) using a simple and inexpensive FIGE technique and that DNA molecules ranging in size from the smallest S. cerevisiae to the largest S. pombe chromosome can be resolved in a single gel using a sequence of only three different field pulses.



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