How to separate chromosomes and restriction fragments using pulsed field gel electrophoresis.

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Background

DNA content of the seven individual *Neurospora crassa* chromosomes ranges from 4 to 10.3 megabases (Orbach 1992). Because of chromosome size, the separation of intact whole-chromosome DNA by pulsed-field gel electrophoresis (PFGE) is slower and more difficult with Neurospora than with yeast (Volrath and Davis 1987) or other fungi that have much smaller chromosomes. Nevertheless, DNAs from the individual Neurospora chromosomes can be resolved successfully. Intact DNA as large as 14 megabases has been separated from one chromosome-rearrangement strain (Barry and Pollard 1993).

PFGE has been used in Neurospora to obtain estimates of chromosome and genome size (Orbach *et al.* 1988, Orbach 1992), to map translocation breakpoints (Smith and Glass 1996), and to isolate DNA from individual chromosomes (Ballario *et al.* 1989, Kelkar *et al.* 2001), thus enabling the generation of physical-clone maps of individual linkage groups by hybridization-based mapping (Aign *et al.* 2001). PFGE has also been used to separate restriction fragments in studies of chromosome breakage and of ribosomal DNA copy-number changes in the nucleolus organizer region (Butler 1991, 1992; Butler and Metzenberg 1990, 1993).

Procedures

Based on earlier electrophoretic karyotyping methodology of Orbach *et al.* (1988), Kelkar *et al.* (2001) describe the equipment, materials, and conditions for the preparation, stepwise separation, and isolation of intact chromosomal DNAs representing the seven linkage groups of *N. crassa*. In wild type, the two largest DNAs (linkage groups I and V) and the two smallest (VI and VII) are too close in size to be well resolved. Separation of these DNAs was therefore accomplished using appropriate translocation strains.

The restriction fragments used by Butler and Metzenberg in their studies were small enough to allow them to follow the protocol used for yeast by Chu *et al*. (1986) and Vollrath and Davis (1987). Electrophoresis was done for 24 hours in 0.5× TBE at 10−11°, with agarose gels cast in 0.5× TBE. Pulse times and voltages were varied to optimize resolution of the pertinent fragments.

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