Yeast artificial chromosomes: rapid extraction for high resolution analysis

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A rapid extraction procedure is described for yeast chromosomes, including yeast artificial chromosomes (YACs) (1, 2). The entire process can be accomplished within 6 hours. Yeast cells were grown to $OD_{600}=7$ in YPD medium, then washed twice in ET buffer (10mM Tris HCl, pH 7.5, 50mM EDTA), and incubated at 37°C in 300μl ET buffer with 0.9M sorbitol and 10μl Novozyme 280L (8800PGU/g Novo Industri, Denmark) for 1 to 2 h. Seaplaque agarose (600μl of a 1% (w/v) solution in 0.125mM EDTA, 10mM Tris HCl, pH 7.5) was mixed with the spheroplasted cells, then pipetted into plug moulds (Bio-Rad). After 20 min at 0−4°C, the plugs were incubated at 60°C in 5% SDS, 10×ET buffer for 2 h. Plugs were incubated at 50°C in 10×ET buffer containing proteinase K (1mg/ml, Merck) and 1% lauryl sarcosine for 2 h, and washed in ET buffer 3×1 minute and stored in 10×ET buffer with 1% lauryl sarcosine at 0−4°C. The plugs produced by this method could generally be stored for at least six months. The chromosome bands (Fig. 1) were sharp and clearly discernible, with little background material, and YACs could be resolved to ±5kb. These methods have been routinely used with thirty-three strains in more than ninety analyses. Apart from laboratory strains of yeast and YAC-containing yeasts this method has been successfully applied to a variety of industrial yeast strains. Because of the shortened preparation time involved CHEF gel electrophoresis could be used to rapidly examine genomic libraries in YACs. The YACs can then rapidly act as a source of YAC DNA for subcloning and radiolabelling with minimal host DNA contamination. Furthermore, by combining rapid chromosomal DNA extraction and high resolution CHEF electrophoresis with other established molecular techniques, utilization and analysis of YACs should be significantly enhanced.

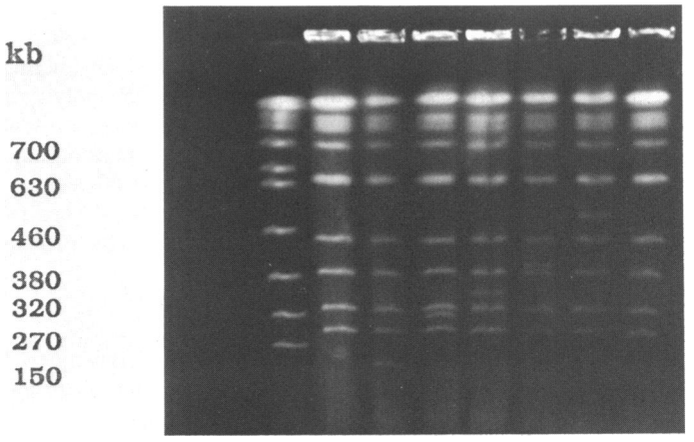

Figure 1. High resolultion CHEF gel of YAC-containing derivatives of AB1380 (D.T.Burke, Princeton University) prepared using the rapid extraction procedure. From left, YNN295 (Bio-Rad), AB1380, MY31 (150kb), MY20 (270kb), MY17 (320kb), MY09 (380kb) and MY32 (500kb), AB1380. Gel was 1.5% agarose (Ultrapure; Clontech), 0.25×Tris-Borate-EDTA, 6 V/cm field strength, 14°C, ramped linearly from 20 to 60 s for 24 h.

REFERENCES

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