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Research Note

FAST PREPARATION OF CHROMOSOMAL DNA FOR PFG ELECTROPHORESIS

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PFGE has become a popular technique for the identification of organisms with industrial or medical relevance, nonetheless its usefulness as a routine methodology is limited by extended and expensive protocols.

In experiments aimed to prepared DNA-protein complexes through PFG, *Leishmania* and yeast cells ($10^8 - 10^9$ cell/ml) were immobilized in low gelling agarose using a 110 ults volume cast mould (Pharmacia) (it is very critical to use a very thin cast mould, and log phase growing cells).

The *Leishmania* cells were treated twice with 0.5% N-lauroylsarcosine sodium salt, 0.5 M EDTA pH 9 at 54 °C for 1 h; at this time, the agarose block should look clear. The samples were sliced (one or more slices are placed per pocket) and loaded in the PFGE apparatus.

The rest of the sample was stored at 4 °C in 0.5M EDTA pH 9. For yeast cells, an 18 h preincubation with 5.000 units/ml of lyticase at 37 °C was included.

As shown in the Figure, there are not differences in number or mobility between the *Leishmania* chromosomal DNA bands prepared by the Fast (lanes 2 and 3) or the traditional procedures (L. H. T. Van der Ploeg et. al., 1984, *Cell, 37:* 7784; G. Carle, & M. Olson, 1986, *Proc. Natl. Acad. Sci. USA, 82:* 3756-3760) (lanes 4 and 5), evenmore, the Fast samples remained stable at least for two months (lane 3). Similar results were found for *Trypanosoma cruzi* and *Crithidia fasciculata* (not shown).

The result for yeast cells was slightly different, i. e., the bands of the Fast (lanes 6 and 7) procedure presented a lower mobility and a minor distortion, probably due to a larger accumulation of cell debris (*Leishmania* has 10 times more DNA per cell than yeast), but the relative band mobility and their number was unchanged, therefore, for most practical purposes, the Fast procedure is equally suitable.

A Western blot experiment, using antibodies against centromeric proteins to assess the fate of DNA-associated proteins, revealed their presence exclusively at the gel pocket, indicating that somehow, the chromosomal bands were stripped of their associated proteins as they advanced into the gel matrix (not shown).

The protocol here presented, is useful specially when a large sample of isolates and small number of cells are handled, as in the characterization of *Leishmania* species (S. Holmes Giannini et al., 1990, *Mol. Biochem. Parasitol., 39:* 9-22) (It is even possible to perform the experiment from primary culture slants).

The Fast procedure, saves nearly 60 h of experimental time in the case of *Leishmania,* and about 50 h in yeast. In addition, ommits the use of Proteinase K, and reduces considerably the use of EDTA.

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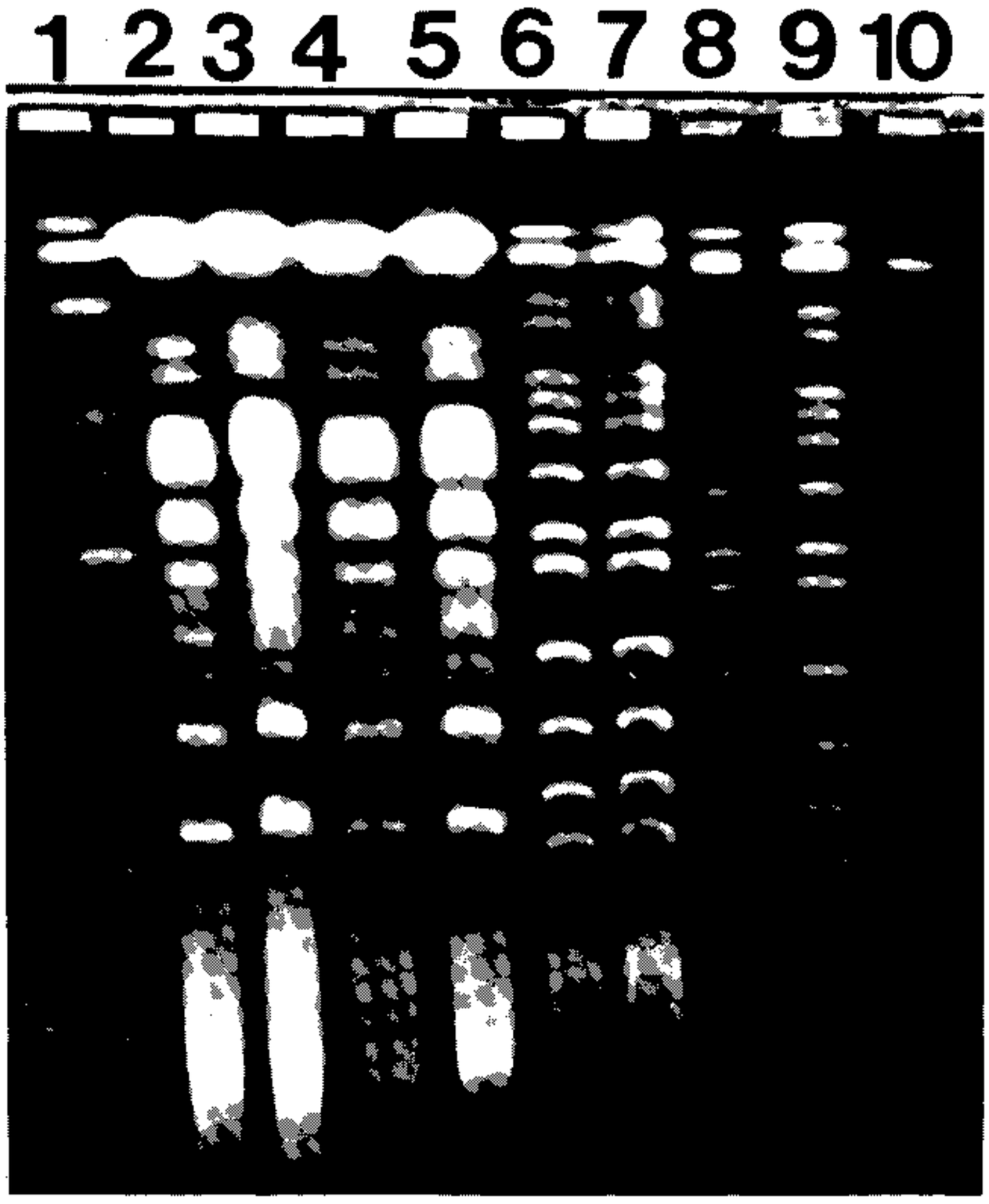

PFG electrophoresis of *Leishmania* and yeast. The cells were prepared by the Fast (F) (lanes 2, 3, 6 and 7) or the regular protocols (R) (the rest), and loaded in a 1% agarose gel. The PFG electrophoresis was performed in a CHEF apparatus at 140 volts, 230 mAmps at 21 °C for 18 h. The cycling interval was performed each 75 sec. with a Programmable Power Electrophoresis Controller PPI-200 MJ Research Inc.
Lanes: 1 and 10 *Saccharomyces cerevisiae* strain AB1380 (R); 2 and 3 *Leishmania mexicana* Bel 21 cells (F) (1/16 and 1/8 of the agarose block respectively), 3 was storaged for 2 months; 4 and 5 *L. mexicana* Bel 21 (R) (1/16 and 1/8 of the sample); 6 and 7 *S. cerevisiae* strain YPH80 (F) (1/16 and 1/8 of the agarose block); 8 and 9 *S. cerevisiae* strain YPH80 (R) (1/16 and 1/8 of the sample).