Temperature gradient increases FIGE resolution

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In pulse field gel electrophoresis, mobility of large DNA molecules is highly dependent on temperature. As they move along a decreasing temperature gradient, their mobilities are progressively reduced. In the FIGE technique (1), this behaviour should cause a sharpening of the bands and should result in a better resolution. It is possible to control temperature in the agarose gel during the electrophoresis by limiting the heat exchange between the gel and the circulating buffer. The gradient is achieved by sandwiching the gel between two bevelled plates.

Two experiments with the settings presented on fig. 1 were run under identical conditions. As can be observed on fig. 2, setting B yielded an improved resolution for the largest chromosomes (2) without altering the resolution for the smaller ones. This increased resolution has been reproducibly obtained with the *S. Cerevisiae* strain CF22. It should be possible for most FIGE apparatus to adapt this modification at low cost.

Figure 1

Methods:

Buffer: 0.5 X TBE

gel: 1% agarose (I.B.F. Ref. A37 NA), 27 cm long, 0.5 cm thick.

electric field: 5 V/cm

ramped period: 30 min (3)

forward/reverse ratio 3:1

forward times (seconds): 3, 6, 9, 12, 15, 18, 24, 30, 36, 42, 48, 54, 60, 69, 78, 87, 96, 108, 120, 132, 144, 156.

Buffer temperature: 6°C

Bevelled plates: 2 mm thick glass plates covered with polymerised silicon seal of decreasing thickness (10 mm to 0 mm).

Figure 2

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