Field inversion gel electrophoresis (FIGE) in vertical slabs as an improved method for large DNA separation

Hugh J.S.Dawkins, Debra J.Ferrier and Terence L.Spencer

Department of Agriculture and Rural Affairs, Regional Veterinary Laboratory, PO Box 388, Benalla 3672, Victoria, Australia
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Field inversion gel electrophoresis (FIGE) has made separation of very large DNA and intact chromosomes a simple operation by utilising conventional submarine agarose gel electrophoresis equipment in conjunction with a switching unit to control the electric field^{1,2}. A vertical slab gel electrophoresis system (Hoefer SE 600, Hoefer Scientific Instruments San Francisco, CA.) was used with frosted glass plates to separate yeast chromosomes and large DNA molecules. TBE buffer (89mM Tris-HCl, 89mM Boric acid, 2mM EDTA, pH 7.8) was used in both buffer chambers and in the preparation of the gel. The buffer temperature was thermostatically controlled by a water bath and pump which circulated water through the heat exchange manifold which incorporates the lower electrode in the SE 600 apparatus. The buffer in the lower chamber was mixed with a stirring bar to ensure efficient heat exchange. Electrophoresis was performed at a constant current of 25 mA for each 160x160x3mm agarose slab gel or at a constant voltage of 60V (3.75V cm⁻¹). FIGE variables were set with an integrated microprocessor controlled switching unit FIGET⁷ (Acronym Pty. Ltd., Mulgrave North, Victoria, Australia). FIGET allows accurate and simple control over the pulse times and ratio of the field inversion as well as the total running time. The FIGET produces a linear progressive increase in the pulse times between the set starting and finishing pulse period with the slope of the ramp being dependent on the total time span of the run. FIGET also provides the options of setting a constant pulse time or a linear decrease in pulse time. A further option is to set a relaxation time between the pulses which is independent of the other functions. Using the technique described, yeast chromosomes, prepared by the method of Carle *et al.*,¹ were separated into 9 bands of which at least three were doublets giving a minimum of 12 chromosomal bands (Lanes A in Figure). These observations are consistent with literature pertaining to yeast chromosomes³⁻⁶. While it remains to characterise the location of particular chromosome bands, the efficiency and clarity of the separations were excellent. Lambda oligomers used as molecular weight markers were also clearly resolved into 18 bands which represents a molecular weight range of 50-900kb pairs (Lanes B in Figure). The conditions for this separation were a switching period which increased linearly from 12 sec. to 90 sec. at a three forward to one reverse field inversion ratio over a 20 hour period. Thus the forward to reverse times began at 9:3 sec and finished at 67.5:22.5 seconds respectively. The temperature in this instance was 14°C although chromosomal separations have been performed at 37°C with excellent results. A constant voltage of 60V (3.75V cm⁻¹) was applied across the gel. The current during the experiment was 25mA falling to 20mA over the 20 hr period. Gel strength was 1% agarose. Advantages of the vertical slab for FIGE when compared with horizontal submarine systems are that the

electrical variables can be controlled accurately and efficiently as all the current must travel through the gel matrix, thus minimising Joule heating effects. As the gels are vertically suspended in 5.5 litres of buffer, the heat exchange potential is enormous, temperature control between 8 and 40°C is simple and also very accurate and the system is not subject to thermal fluctuations. These features offer clear advantages over the current submarine systems for large DNA molecule separations which use very elaborate temperature control systems^{3,4} due to the high voltages and currents which are applied across the gels. Further advantages offered by vertical gel FIGE are that 2-4 gels can be run simultaneously, under identical conditions. Agarose gel gradients and wedge gels, which may offer improved separation and resolution can also be tested in this system. The choice of spacers allows the preparation of gels of defined thickness. This technique is recommended for large DNA separation in which reproducible and accurate control over the conditions of temperature and current flow through the gel matrix are required and is ideal for processing large numbers of samples simultaneously.

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7. FIGET is a registered name of Acronym Pty Ltd, P.O. Box 322, North Mulgrave 3170 Victoria Australia.