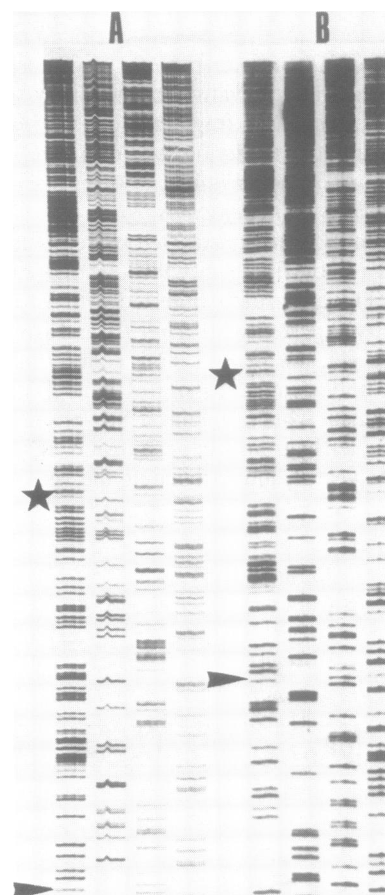
A simple discontinuous buffer system for increased resolution and speed in gel electrophoretic analysis of DNA sequence

Piero Carninci, Stefano Gustincich, Steve Bottega, Cristina Patrosso[1], Giannino Del Sal, Guidalberto Manfioletti and Claudio Schneider*

International Center for Genetic Engineering and Biotechnology, 99 Padriciano, 34012 Trieste and [1]Istituto Tecnologie Biomediche Avanzate, via Ampere, 56 Milan, Italy

Submitted November 23, 1989

The standard sequencing gel system using Tris/Borate/EDTA does not allow separation of DNA fragments >300nts. albeit with the use of long gels and/or longer running time. We have used the discontinuous buffer system first introduced by Allen (1): it uses Tris-sulphate/leading anion as running gel buffer and Tris-borate/trailing anion as tank buffer. We have obtained: a) increased sharpening of bands with consequent enhanced resolution b) increased resolution in the >250nt. region (1.4 fold relative magnification) c) decreased running times. For 1 liter 5× stock solution of running gel buffer use 9.8 ml of concentrated (96%) H_2SO_4 and 378 g. Trizma base (pH 9.0). For 1 liter 5× stock solution of tank buffers use 43.5 g. Boric acid and 435 g. Trizma base (pH 9.0). We use 0.2 mm thick/40 cm. long/20 cm. wide gels (2); for 30 ml of gel mixture add 15 g. urea (8 M final), 6 ml of running gel buffer (5×), relative Acrylamide/Bis amount (from 30% Acrylamide/1% Bis stock solution), double distilled water to 30 ml, 30 μl of TEMED and 60 μl of APS (25%). Sequencing reactions were performed using the Pharmacia T7 DNA polymerase kit using 35^{S}-dATP and single stranded DNA template. A: sequence analyzed on a 6% acrylamide gel using the discontinuous system (135 minutes 1800 V/25 mA/35 W constant). B: the same sequence as in A analyzed using TBE buffer on a 6% acrylamide gel, same running conditions as for A (arrow=245nts, star=400nts from the primer). We are confident that due to the increased band sharpness, increased migration velocity with consequently decreased running time and overall increased resolution power the described discontinuous buffer system is a simple and advantageous alternative to the more familiar TBE buffer.



ACKNOWLEDGEMENTS

This work has been funded by CNR 'Progetto Strategico Genoma Umano' and AIRC 'Associazione Italiana per la Ricerca sul Cancro'.

REFERENCES

1. Allen,R.C., Graves,G. and Budoule,B. (1989) *Biotechniques* **7**, 736–744.
2. Ansorge,W. and Garoff,H. (1981) *Analyt. Biochem.* **115**, 450–457.

* To whom correspondence should be addressed