Volume 15 Number 7 1987 Nucleic Acids Research

PFGE of human DNA: 5-azacytidine improves restriction

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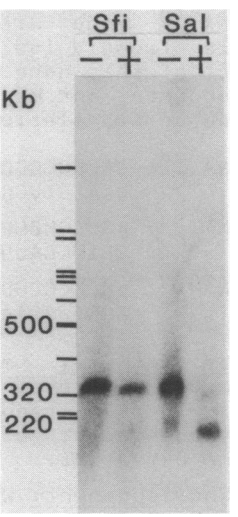
Submitted February 24, 1987

Pulsed field gel electrophoresis (PFGE) (1) of human DNA and the development of long-range restriction maps are frequently hampered by the cytosine methylation that occurs at CG dinucleotides in human DNA. This methylation can interfere with restriction and change the size, number and concentration of fragments detected in Southern blots. We have found that these effects, which vary at different loci and in different cell lines, can be partially overcome by growing cells in 5-azacytidine (5-aC) prior to DNA isolation. This treatment increases the number of enzymes that can be used to map a locus; it can help distinguish between polymorphic methylation patterns and polymorphic restriction patterns; and it can establish distinguishing characteristics for particular restriction fragments.



Exposure to 5-aC reduces the level of methylation(2). We found that after two weeks of growth in 2-5 μM 5-aC (Sigma), human lymphoblastoid DNA was digested to a smaller average fragment size by Mlu I, Nru I, Sal I and Sma I. This change was accompanied by changes in the Southern blot pattern of particular loci. The figure compares the Sfi I [GGCC(N_5)GGCC] and Sal I [GTCGAC] digestion of the X chromosome locus DXS105(3) in DNA from untreated (-) and from 5-aC-treated (+) cells. Sfi I is rarely affected by methylation at CG and the 350 kb Sfi I fragment was unchanged by 5-aC treatment. In contrast the 350 kb Sal I fragment in the untreated DNA was further digested to 180 kb in the 5-aC treated DNA. (The approximate position of yeast chromosome markers is indicated on the left). The 5-aC treatment is effective for the enzymes mentioned above and particularly for Sal I. In addition, Not I, BssH II, Apa I, Sst II and occasionally Sfi I may also be affected at specific loci. (Our experience suggests that 5-aC may not be stable in solution for more than 3 weeks).

References
1. Schwartz, D.C., Cantor, C.R. (1984) Cell, 37:67-75.
2. Jones, P.A. and Taylor, S.M. (1980) Cell, 20:85-93.
3. Hofker, M.H. et al. (1985) Cytogenetics and Cell Genetics 40:652.