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[54] METHOD AND APPARATUS FOR DETECTING SINGLE BASE MUTATIONS IN DNA WITH DENATURING GRADIENT ELECTROPHORESIS

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[58] **Field of Search** 435/6, 291, 300, 310, 435/40, 173, 289, 290, 803, 973; 436/501, 809; 935/19, 78, 86

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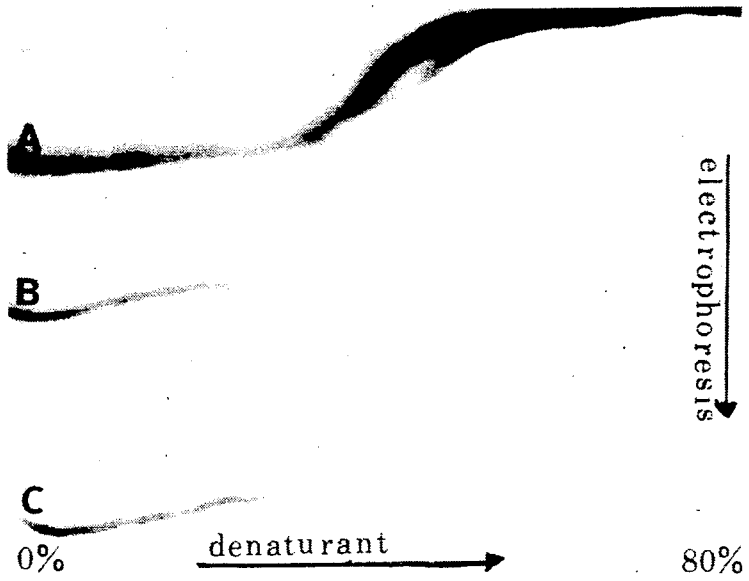
[57] **ABSTRACT**

Single base pair differences between otherwise identical DNA fragments can result in an altering of the melting behavior which can be detected by denaturing gradient gelelectrophoresis (DGGE). A method has been developed for efficient transfer of genomic DNA fragments from the gel following DGGE. The DGGE is run in a polyacrylamide gel (PAG) containing 2% low gelling temperature agarose (LGT). The PAG is crosslinked with a reversible crosslinker, and after electrophoresis the crosslinks are cleaved and 80–100% of the DNA fragments are transferred to nylon membranes by alkaline transfer. Hybridization with gene specific probes is then performed. The technique has been used to identify an RFLP in the COLIA2 gene.

9 Claims, 5 Drawing Sheets