

PRELIMINARY STUDIES WITH A FLUORESCENT DETECTION
PROCEDURE FOR DNA HYBRIDATION

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Nucleic acid hybridisation is a powerful technique in molecular biology, having widespread applications in various fields, particularly in diagnostic medicine.

In view of the disadvantages of using radio labelling methods in nucleic acid hybridization assays, alternative non-radioactive detection methods have been developed such as the biotin-Avidin-Alkaline phosphatase (AP) system. Here we describe a modification of the above system using a fluorogenic substrate 4-methyl umbelliferyl phosphate (4 MUP) for alkaline phosphatase.

Serial dilutions of Setaria digitata DNA (100ng - 10pg) were immobilised on nitrocellulose and filters were then probed with either ^{32}P -dCTP or bio-21-dUTP nicktranslated S. digitata specific DNA probe pSD14. Biotinylated filters were either developed using a commercially available kit or using 4MUP as substrate. The ability of the 4MUP system to detect bands in a Southern blot was also determined. Reactions involving 4MUP were visualized using a UV transilluminator. The sensitivity of the method (using 4MUP) was comparable to that of the isotopic and colorimetric methods and has the added advantage over the colorimetric method in that the nitrocellulose filter could be reused after deprobing.

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