

0240 NON-ISOTOPIC DETECTION TECHNIQUES FOR SOUTHERN BLOT ANALYSIS OF SINGLE COPY GENES, Stavroula Hassapoglidou, Eleftherios P. Diamandis (Dept. Clin. Biochem., The Toronto Hospital and Univ. of Toronto, Toronto, Ontario, Canada)

Nucleic acid hybridization is a powerful technique which is now used frequently for research purposes and routine testing. Until recently, the label of choice for such assays was ^{32}P which has a short half-life, it poses disposal and safety problems and necessitates long exposure times of many hours to many days. We compared the ability of ^{32}P -based assays and some non-isotopic detection systems to detect single-copy genes on Southern blots. We used a raf-1 protooncogene probe cloned into the expression vector pSP65. Probe was either DNA or RNA labeled with ^{32}P , digoxigenin or biotin. DNA was labeled with the random primer method and [α - ^{32}P] dCTP, digoxigenin-11-dUTP or biotin-11-dUTP. RNA probes were produced by use of SP6 RNA polymerase and [α - ^{32}P] UTP, digoxigenin-11-UTP or biotin-21-UTP. Digoxigenin and biotin were then linked to alkaline phosphatase (ALP) with use of anti-digoxigenin antibodies or streptavidin, labeled with ALP, respectively. ALP activity was detected with a chromogenic substrate (BCIP/NBT), a chemiluminogenic substrate (AMPPD) or a fluorogenic substrate (3-hydroxy-2-naphthoic acid-2-phenylamido phosphate, HNPP). Human DNA was extracted from leucocytes and restricted with one of the enzymes EcoRI, Hind III and PstI. Southern blots were prepared with variable loadings of total genomic DNA from 1 to 10 $\mu\text{g}/\text{lane}$. Our results suggest the following: RNA probes were superior to DNA probes in terms of sensitivity and specificity; digoxigenin was superior to biotin in terms of sensitivity and levels of background signals. The three substrates tested have similar detectabilities when optimized incubation times are used i.e. 16-24h (BCIP/NBT), 2h (HNPP) or 20 min-1 h (AMPPD). The AMPPD method affords very short incubation and exposure times but background signals vary. Best background signals were observed with the ^{32}P -method.