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Dot blot detection of point mutations with adjacently hybridising synthetic oligonucleotide probes

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Due to non specific hybridisation to high molecular weight DNA, detection of point mutations in human DNA with oligonucleotide probes usually requires digestion with restriction enzymes and gel electrophoresis. We have devised a simple method which enhances the hybridisation specificity of oligonucleotide probes such that analysis by "dot-blots" can be undertaken.

This method has been applied to detection of the activating mutation in the Ha-ras gene in human T24 bladder carcinoma cells (2). 20ug DNA from normal peripheral blood (N) or T24 cells was digested with SacI, subjected to electrophoresis on a 1% agarose gel and the gel was subsequently dried (1). Gels were prepared and subjected to hybridisation as described elsewhere (1). A ^{32}P labelled oligonucleotide probe with the sequence $^{5'}$pCCCACACCGACCGC-GCCCAC$^{3'}$ was prepared by 3' primer extension (1) using ^{32}P dCTP. This was used for hybridisation at 10ng/ml and 70°C (Td-2°C) together with 10ng/ml of the adjacently hybridising oligonucleotide $^{5'}$CAGCTGGATGGTCAGCGCACTCTTG$^{3'}$. Following hybridisation for 2 hours, gels were washed for 4 x 5 minutes in 6 x SSC and then at 70°C for 2 minutes. Autoradiography at this stage (2 days) revealed a significant high molecular weight hybridisation signal from both normal and T24 DNA (see figure) which, with dot blot analysis, masks the specific signal from the activated Ha-ras oncogene. Parallel gels were soaked in PBS containing 2% bovine albumin (Miles) and 0.1% Trion-X-100 for 30 minutes and then washed 5 times in PBS/2% bovine albumin. Gels were then immersed in ligase solution (0.3 units/ul T4 DNA ligase from Boehringer in the recommended ligation buffer) for 80 minutes at 16°C before washing in 6 x SSC at 75°C for 10 minutes. Gels were then autoradiographed for 2 days. This additional ligase treatment resulted in high temperature removal of the background high molecular weight hybridisation signal and a specific dot blot hybridisation of the probe to T24 DNA alone. Omission of the ligase leads to total loss of hybridisation signal. This method simplifies the detection of point mutations in human DNA and provides a convenient alternative to amplification of target DNA (3).

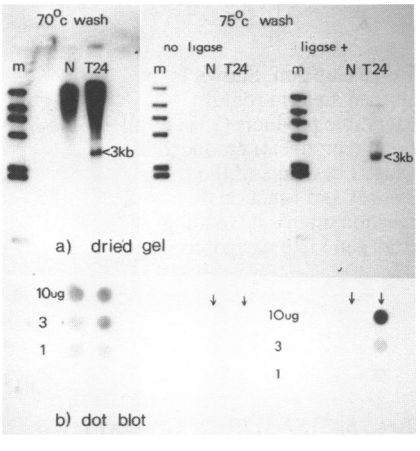

Figure 1: Dried gel (top) or dot blot (bottom) hybridisation of T24 activated Ha-ras specific oligonucleotide probe under standard conditions (70°C wash) or following treatment with T4 DNA ligase and washing at 75°C. m -λ HindIII marker.

References:

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