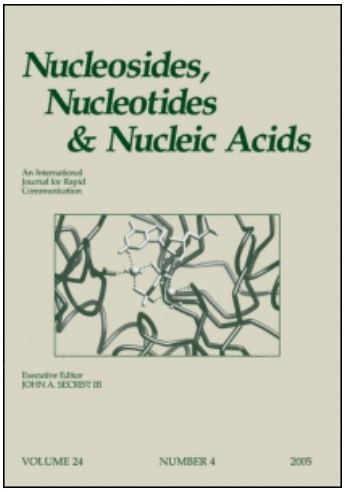

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OLIGONUCLEOTIDE-POLYAMIDE CONJUGATE PROBES WITH MULTIPLE NON-RADIOACTIVE LABELS: APPLICATIONS AND COMPARISON TO RADIOLABELLED PROBES

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Abstract. Oligonucleotide-polyamide conjugate molecules containing multiple biotin labelled polyamide have been used as non-radioactive probes. We found that these probes have similar sensitivity to ^{32}P labelled probes. Chemiluminescent detection is the method of choice.

We have reported recently the preparation and use of oligonucleotide probes containing a pendant polyamide moiety on the 3' end.[1,2] The probe used in the current study was prepared as described previously, except that the first amino acid was added as the Fmoc derivative. The biotin residues were added after oligonucleotide assembly, prior to cleavage of the conjugate from the solid support. The structure of the probe is d(GGGCTTCACAACATCTGTGATGTCAGCAGG)p-$O(CH_2)_3C(O)$-[εAhx-Lys(Biotin)-]$_{10}$Ala. The oligonucleotide is KPIB, complementary to a region of mouse mRNA that is common to all the mouse kallikreins, a series of processing enzymes[2]. The target DNA is a plasmid with a mouse kallikrein cDNA insert. The negative control is a plasmid containing an unrelated insert. Two different detection systems were tested: the NBT/BCIP system previously described[2] and the Photogene system from Life Technologies Inc., Gaithesburg, U S A.

Figure 1 shows the results obtained on dot blots using the various detection procedures. The strip on the left shows detection using a normal (non-biotinylated) KPIB probe labelled with ^{32}P at the 5' end by T_4 polynucleotide kinase. The middle strip shows the result obtained when the biotinylated probe was detected using the Photogene kit and the strip on the right detection by the NBT/BCIP procedure. The limits of the ^{32}P and Photogene detection are comparable; they are both approximately 5 pg of plasmid (2 attomole). The NBT/BCIP detection appears to be slightly less sensitive, at 20 pg of plasmid.

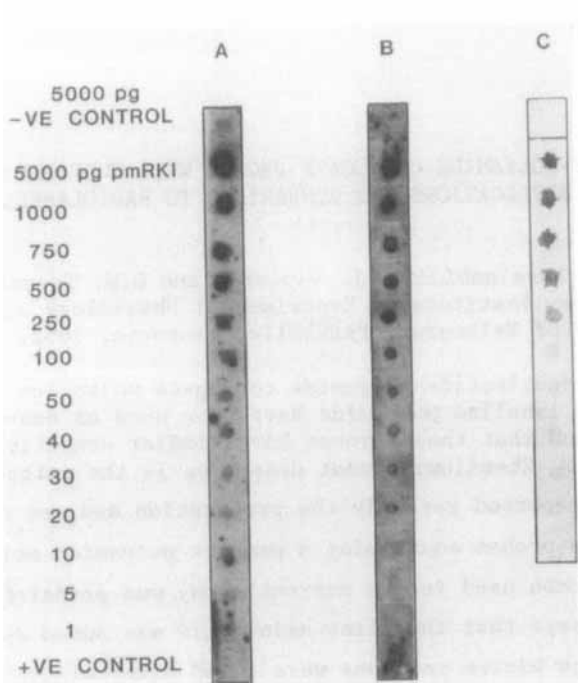

FIGURE 1. *Detection of kallikrein containing plasmid using KPIB probe. Lane A, ^{32}P detection; lane B, chemiluminescent detection; lane C, NBT/BCIP detection. The ^{32}P detection was using a normal (unbiotinylated) ^{32}P end-labelled probe, with overnight detection with an intensifying screen. The biotinylated probe used in lanes B and C was KPIB-[εAhx-Lys(Biotin)-]$_{10}$ Ala. The chemiluminescent detection was carried out for 10 min, starting at 45 minutes after the addition of the chemiluminescent substrate. The NBT/BCIP detection was overnight. Nitrocellulose membranes were used for the ^{32}P and NBT/BCIP blots, whereas nylon membrane was used in the chemiluminescence experiment. Only the middle (chemiluminescent) strip has a positive control as the last dot. The first dot in lane C is 2000 pg.*

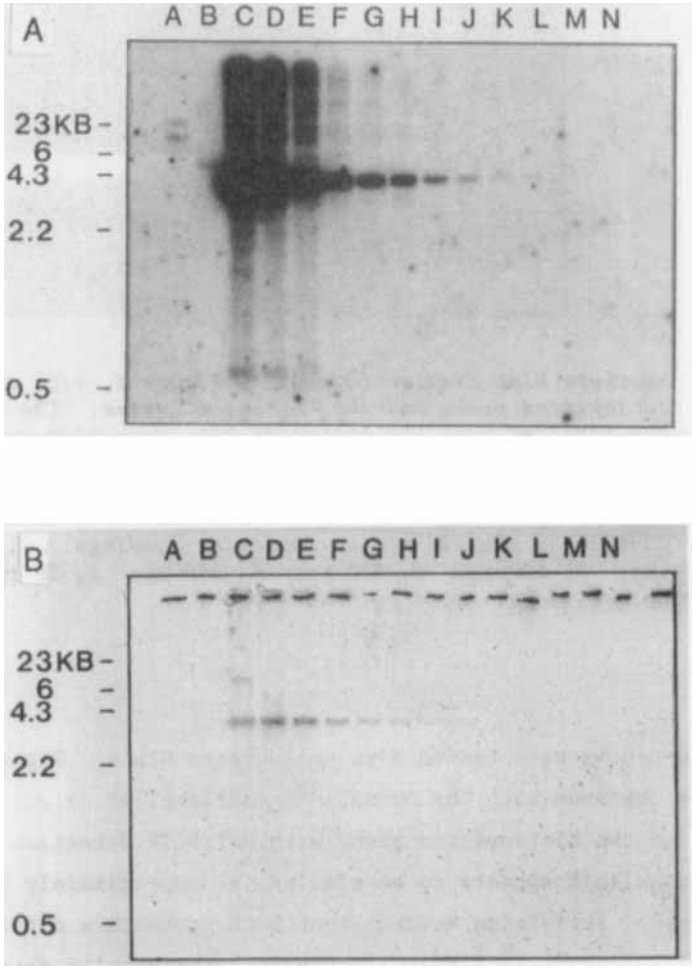

FIGURE 2. *Southern blots of the 3.7 kb plasmid containing a kallikrein insert, detected with a normal ^{32}P end-labelled probe (panel A) and with the same biotinylated probe as in Figure 1, with NBT/BCIP detection (panel B). Both blots are on nitrocellulose paper. Lane A, 200 ng of Hind III λ markers; lane B, 20 ng of negative control plasmid (Gemini plasmid with an unrelated insert, 5.1 kb total); lanes C-N contain a 3.7 kb plasmid with a kallikrein insert: C, 20 ng; D, 10 ng; E, 5 ng; F, 1 ng; G, 500 pg; H, 250 pg; I, 100 pg; J, 50 pg; K, 20 pg; L, 10 pg; M, 5 pg; N, 1 pg. Detection is overnight in both cases, and an intensifying screen was used in the case of ^{32}P detection.*

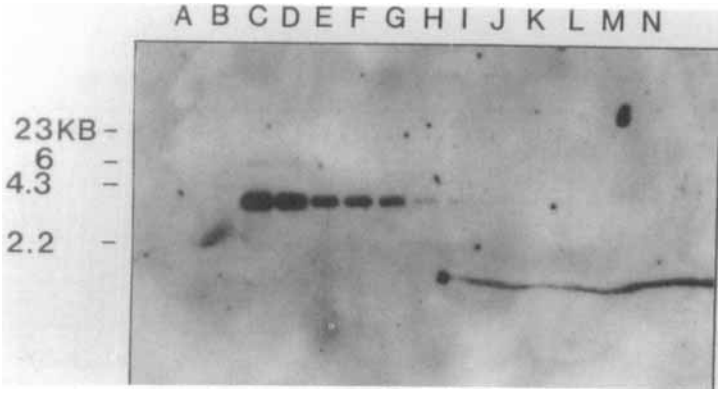

FIGURE 3. *Southern blot similar to that in Figure 2, with detection using the biotinylated probe and the Photogene system. The blot was on nylon membrane supplied with the Photogene kit, detection on Kodak XAR film for 15 min, 80 min after the addition of the chemiluminescent substrate. Note that the loadings start at 10 ng in this blot. Thus, lane B contains 10 ng of negative control plasmid and lanes C-N the kallikrein containing plasmid at the following loadings: C, 10 ng; D, 5 ng; E, 1 ng; F, 500 pg; G, 250 pg; H, 100 pg; I, 50 pg; J, 20 pg; K, 10 pg; L, 5 pg; M, 4 pg; N, 3 pg.*

These probes were tested also on Southern blots. Figure 2 shows the results obtained with the normal ^{32}P end-labelled oligonucleotide (panel A) and the biotinylated probe with NBT/BCIP detection (panel B). The detection limit appears to be similar, at approximately 20 pg (lane K) of plasmid. It is also evident that both probes are detecting the correct size fragment (3.7 kb). In Figure 3 the results obtained when using the Photogene system for the detection of the biotinylated probe are shown. The result is similar to those in Figure 2; a limit of approximately 20 pg of plasmid (lane J) is detected.

In summary, the use of oligonucleotide-biotinylated polyamide conjugate probes gives similar levels of sensitivity to normal 5'-^{32}P end-labelled oligonucleotide probes. In particular, the chemiluminescent detection is attractive with a much shorter detection time (10 min exposure) than ^{32}P or NBT/BCIP (16 hr). The results obtained with the chemiluminescent detection of the biotinylated probe are also more durable, because an exposure on an X-ray film is obtained, in contrast to the coloured reaction product in the case of the NBT/BCIP

detection, which is liable to fade on continued exposure to light. Therefore, the use of these biotinylated probes with chemiluminescent detection offers the advantages of ^{32}P labelled oligonucleotides, with similar sensitivity but shorter detection time.

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