CDP-STAR™ AS A CHEMILUMINESCENT SUBSTRATE FOR USE WITH ALKALINE PHOSPHATASE LABELLED PROBES

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INTRODUCTION

In the last five years alkaline phosphatase-labelled probes have been widely adopted for use in DNA fingerprinting and DNA profiling eg NICE™ probes (Cellmark) (Ref. 1). Apart from the obvious operational advantages of using non-radioactive systems, hybridisation with alkaline phosphatase-labelled probes is far more rapid than with traditional systems. Typically membranes can be hybridised and autoradiographs produced within seven hours. Hybridisation is detected by the application of a chemiluminescent substrate to the membrane. The substrate is activated by the alkaline phosphatase label on the probe resulting in emission of visible light which is recorded on X-ray film. One of the slowest parts of the procedure is exposure of hybridised membranes to X-ray film, which takes 3-4 hours or more. In an effort to shorten exposures and reduce the overall length of the DNA profiling process, we examined the performance of two different chemiluminescent substrates, Lumi-Phos® 530 (Lumigen Inc, Ref. 2) and CDP-Star™ (Tropix Inc, Ref. 3) which is reported to give shorter exposure times.

METHODS

Membranes (Hybond-N, Amersham) containing HinfI digested human DNA were hybridised to various NICE™ probes (Cellmark) following recommended conditions. These were either standard membranes containing concentration gradients of K562 DNA (Fig. 1 and 3) or samples from paternity casework at approximately 1-4 μg per track (Fig. 2). Following hybridisation and washing, membranes were either sprayed evenly with Lumi-Phos® 530 or incubated in CDP-Star™ for 5 minutes at room temperature. Each membrane was then sandwiched between two polyester sheets and excess chemiluminescent substrate gently squeezed out. Exposure to X-ray film (Hyperfilm, Amersham) was carried out at 30°C for various lengths of time.

RESULTS

Figure 1 shows two identical membranes containing HinfI-digested K562 genomic DNA and probed with MS31. The membrane in Fig 1a was treated with CDP-Star™ and exposed to X-ray film for 1 hour while that in Fig 1b was treated with Lumi-Phos® 530 and exposed to X-ray film for 1 hour. After a 1 hour exposure, 10ng of DNA was clearly visible on the CDP-Star™ treated membrane, while on the Lumi-Phos® 530 treated

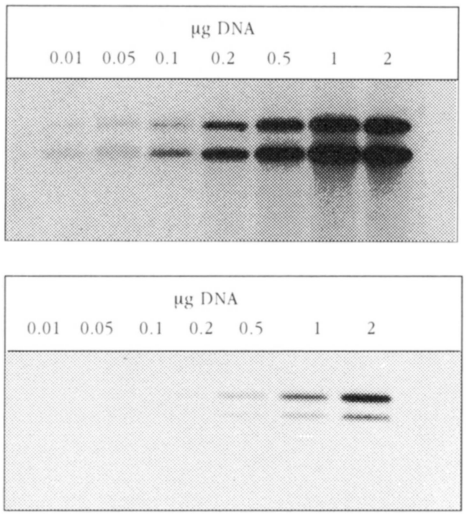

Figure 1a, b
Gradients of HinfI digested K562 DNA probed with MS31.
a CDP-*Star*™ treated and exposed to X-ray film at 30 °C for 1 hour.
b Lumi-Phos® 530 treated and exposed to X-ray film at 30 °C for 1 hour.

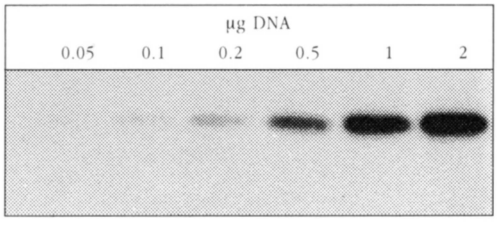

Figure 3
Membrane containig a gradient of HinfI digested K562 DNA, probed with G3 and treated with CDP-*Star*™ which had been used on the previous 3 days. Exposure was for 1 hour at 30 °C.

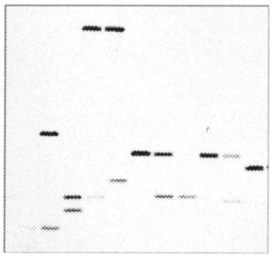

Figure 2a

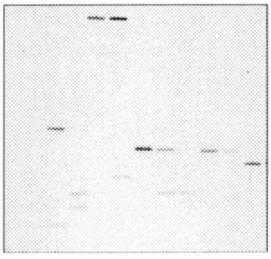

Figure 2b

Figure 2a,b
Human DNA samples digested with HinfI, probed with MS205 and exposed to X-ray film for 30 minutes at 30 °C.
a 4 hours after CDP-Star™ treatment.
b 2 days after CDP-Star™ treatment.

membrane 200 ng could be detected.

In order to examine the flexibility of using CDP-Star™, a membrane containing paternity casework samples (approximately 1-4 µg DNA per track) was treated with CDP-Star™ and exposed to X-ray film after 4 hours (Fig. 2a), and then again 2 days after CDP-Star application (Fig. 2b). Chemiluminescence of CDP-Star™ continues over a period of several days and although sensitivity of detection does decrease with time, several exposures of a membrane can be taken without the necessity of reprobing or reapplication of chemiluminescent substrate.

The potential for reuse of CDP-Star solutions is shown in Figure 3. A membrane containing a concentration gradient of K562 DNA was hybridised with NICE™ probe G3 and incubated in CDP-Star™ which had already been used on the three preceding days and stored at 4°C. Following a 1 hour exposure, 100 ng of K562 DNA is still clearly visible.

DISCUSSION

Various chemiluminescent substrates are now available for use with alkaline phosphatase labelled probes, all offering major advantages over the traditional radioactive systems. This study compares the performance of CDP-Star™ and Lumi-Phos® 530 in DNA Profiling. Whilst the overall sensitivity (ie total chemiluminescence) of both substrates is very similar, the use of CDP-Star™ reduces film exposure time significantly. This offers clear advantages in DNA profiling as sequential hybridisation with several probes can take several days to accomplish. It is also important in forensic applications where very small DNA samples can often only be visualised after lengthy exposures.

REFERENCES

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