# A Non-Isotopic DNA Detection System With the Sensitivity of $^{32}P$: Applications For Paternity and Forensic Identifications

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## INTRODUCTION

DNA blot hybridization is used for identity testing because the DNA of every individual (except for identical twins) is unique. In forensic casework where the amount of material is limited, a sensitive detection system such as $^{32}P$ must be employed to discern variable number of tandem repeat (VNTR) polymorphisms in the sample. However, $^{32}P$ labeled probes have numerous shortcomings including: a short biochemical half life, and the requirements for safety shielding, constant monitoring and a radioactive license. The short biochemical half life effectively means that fresh probe must be labeled once a week, thereby creating a possible quality control problem of lot-to-lot variation. To avoid these problems, we have developed a non-isotopic system for paternity and forensic identity testing.

## EXPERIMENTAL AND DISCUSSION

The non-isotopic system utilizes an alkaline phosphatase conjugated DNA probe to hybridize specifically to VNTR's in the sample. The probe is detected using a phosphorylated 1,2-dioxetane chemiluminescent substrate (Bronstein and Voyta 1989; Schaap et al. 1989). When the substrate is dephosphorylated by the alkaline phosphatase labeled probe, light is emitted at 470 nm. The light is localized to the site of the reaction and can be detected on x-ray film. Light is steadily emitted by the enzyme/substrate reaction for at least 6 days (data not shown).

Alkaline phosphatase labeled probes in a chemiluminescent system detect the same VNTR sequences as equivalent probes labeled with $^{32}P$ (Fig. 1). An example of a paternity inclusion and an exclusion using two different probes is shown. In these cases, the chemiluminescent signal can be detected in 2 hours from 1 $\mu$g of genomic DNA with total hybridization and detection times of 3 to 5 hours. Similar results with a $^{32}P$ detection system require longer exposure times (Fig. 1).

The sensitivity of the chemiluminescent system was examined by hybridizing an alkaline phosphatase labeled probe to varying amounts of genomic DNA. The probe to the D2S44 locus easily

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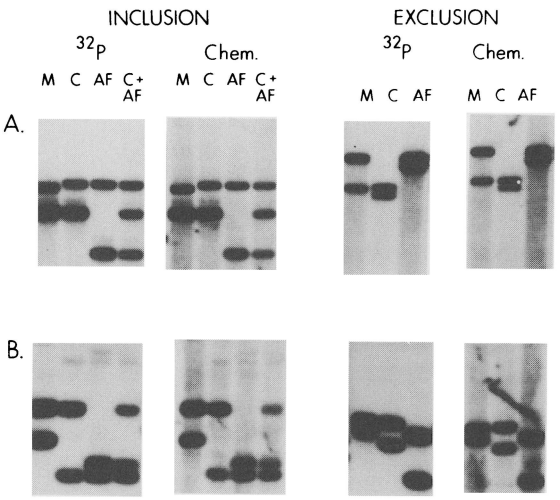


Fig. 1. A comparison of the $^{32}P$ and chemiluminescent detection systems for paternity identifications. One $\mu$g of DNA from each individual was digested with Pst I, separated on a 1% agarose gel, and transferred to an MSI Magna Nylon membrane. The DNA was from the mother (M), child (C), alleged father (AF), or a mixture of child plus alleged father (C+AF). The blots were hybridized with oligonucleotides to the D2S44 (A) and D17S79 (B) loci. Each of the cases was done on duplicate blots using either $^{32}P$ labeled probes or probes directly coupled to alkaline phosphatase (Chem.) as indicated. The alkaline phosphatase labeled probes were detected using a phosphorylated chemiluminescent substrate (Bronstein and Voyta 1989; Schaap et al. 1989). The $^{32}P$ detection system was exposed for 16 hours to x-ray film and the chemiluminescent system was exposed for 2 hours.

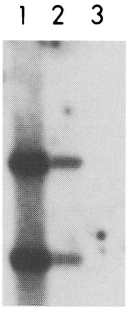


Fig. 2. A genomic DNA titration showing the sensitivity of the chemiluminescent detection system. 400 ng, 40 ng, and 4 ng of Hae III digested human genomic DNA (lanes 1-3) was separated on a 1% agarose gel and hybridized with alkaline phosphatase labeled probe to the D2S44 locus. The blot was exposed to x-ray film for 16 hours.

detected the VNTR sequences from 40 ng of DNA (Fig. 2) which is equivalent to the amount of DNA found in 1-2 $\mu$l of blood. On the original autoradiograph, the probe could weakly detect the VNTR pattern from 4 ng of DNA which is similar to the results obtained with $^{32}P$ labeled probes. This high sensitivity is required for forensic samples because they are often degraded.


Fig. 3. The assembled components of a chemiluminescent identity test. Lane 1 contains the Lifecodes sizing standard. Lane 2 contains 1 μg of Pst I digested human genomic DNA. The blot was hybridized with alkaline phosphatase labeled oligonucleotides complementary to part of the sizing standard and to the D2S44 and D17S79 loci.

The Lifecodes $^{32}P$ identity test consists of two labeled probes and a set of molecular weight markers on each blot. Figure 3 shows preliminary results of an equivalent chemiluminescent test thus confirming that all the components of a $^{32}P$ identity test can be assembled into a chemiluminescent test. The extra bands in the middle of lane 2 have been shown to result from non-optimal stringency conditions for the membrane wash. This locus presented similar challenges while optimizing stringency conditions for the $^{32}P$ based procedure.

Three important advantages of the chemiluminescent detection system are that it requires few changes in the existing identity test format, the probes have been shown to be stable for >1 year, and it is extremely sensitive. VNTR signal can be detected from <40 ng of human genomic DNA which is the level required for forensic samples. Other non-isotopic systems work well for paternity testing (Dykes 1988) but do not have the required sensitivity for forensic casework.

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