USE OF CHEMILUMINESCENT LABELED PROBES FOR FORENSIC AND PATERNITY DETERMINATIONS.

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Introduction.

Since the middle 1980's, issues involving human identification in forensic or paternity cases have been addressed by RFLP DNA analysis using probes radiolabeled with P^{32} (Kanter et al., 1986; Guisti et al., 1986; Baird et al., 1986). The main disadvantage of these types of probes are their short shelf life and the general inconvenience associated with working with radioactive materials. For the past several years our laboratory has been involved in the conversion of these probes to a non-isotopic format. This has been accomplished through the development of oligonucleotide probes labeled with alkaline phosphatase (AP-probe) that recognize VNTR loci (Baum et al. 1990, Neuweiler et al. 1992). The final detection of AP-probes is based on the detection of chemiluminescence with the substrate, adamantyl 1,2-dioxetane phenyl phosphate (Lumiphos™). This detection system offers equivalent sensitivity to P^{32} allowing detection of genomic DNA in the ng range.

Materials and Methods

Standard RFLP methodology was utilized to prepare, digest, size separate and transfer DNA. Starting with DNA bound to dry nylon membranes, the general procedure was as follows:

Wet Membranes (5 to 10 min at room temp.)
Hybridization (20 min at 55 °C)
Wash I (2 times for 10 min each at 55 °C)
Wash II (2 times for 10 min at 55 °C)
Rinse with Quick-Light™ Buffer (4 x 1 min dip)
Spray substrate (Lumi-Phos™)
Expose to X-ray film (O.N. at room temp. or 1-6 hrs at 37 °C)

Results and Discussion

Numerous VNTR loci were tested for their utility for DNA typing and for the properties of their respective AP-probes. Each AP-probe was synthesized as an oligonucleotide and directly conjugated with AP. Table 1 provides a list and brief description of the properties of 14 loci available in our laboratory for paternity and/or forensic DNA profiling. Where appropriate, databases have been developed with Hae III and/or Pst I digested DNA from unrelated North American Blacks and Caucasoids individuals. In addition, for some loci, databases have been generated from 14 different ethnic groups (Balazs, 1993).

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Table 1. **Properties of AP-probes used for the analysis of VNTR loci.**

SIZE RANGE OF DNA FRAGMENTS (KB)				
		Restriction Enzyme		%
Locus	Hae III	Hinf I	Pst I	Heterozygosity
D1S7	1.6 - 23	0.8 - 25		99
D1S339	0.6 - 12	1.2 - 13	1.2 - 13	95
D2S44	0.2 - 11	1.5 - 10	6.8 - 16	96
D2S92	2.4 - 13			94
D4S163	1.3 - 16	1.2 - 12	3.6 - 16	95
D6S132	1.0 - 8.5	4.3 - 12	1.7 - 9	92
D7S21		3.0 - 20	4.5 - 20	97
D7S467	1.6 - 7		3.5 - 15	94
D10S28	0.2 - 13			94
D12S11		3.0 - 18	4.7 - 25	95
D16S85	0.4 - 7	0.3 - 9	0.6 - 10	92
D17S79	0.6 - 2.4	1.0 - 4.3	2.5 - 5.7	88
D18S27		1.0 - 6.2	4.0 - 9	92
DNF24		1.0 - 23	2.0 - 25	multilocus

The use of AP labeled probes has a number of advantages over traditional radiolabeled probes. These advantages include:

1. Stability
   AP-labeled probes are stable for at least one year.

2. Safety
   AP-labeled probes do not require containment or monitoring because they are not radioactive. The reagents utilized do not require special handling.

3. Hybridization
   The hybridization conditions are generally milder (55 °C vs 65 °C) and shorter (20 min vs several hours) resulting in a decreased loss of DNA from filter. Exposures to the X-ray film are at room temperature or 37 °C instead of

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70 °C. Because light emission lasts for up to 3 days, re-exposures are easily obtained. The resulting images are sharper than those obtained with ^{32}P-labeled probes, therefore AP-probes give better resolution and greater power of exclusion.

4. Speed
   Because of the fast and simple protocol it is possible to obtain at least one set of results per day. By doing daily rehybridizations with various AP-probes, it is easy to produce results with four different probes in a one week period.

5. Rehybridization
   The AP-labeled probe is inactivated by heating at 65 °C for 20 min. Hybridization of membranes can be readily interchanged between AP-probes and radioactive probes. Therefore, rehybridization can be performed with ^{32}P-labeled or AP-labeled probes.

In summary, the use of AP-labeled probes is faster, safer, and easier than radiolabeled probes. They are ideal for laboratories that want to move away from the use of radioactive materials and/or without the resources for radiolabeled containment or for laboratories interested in fast turnaround time.

REFERENCES

Baird M, Balazs I, Guisti A, Miyasaki L, Nicholas L, Wexler K, Kanter E, Glassberg J, Allen F, Rubinstein P, Sussman L (1986) Allele frequency distribution of two highly polymorphic DNA sequences in three ethnic groups and its application to the determination of paternity. Am J Human Genet 39:489-501.

Balazs I. (1993) Population genetics of 14 ethnic groups using phenotypic data from VNTR loci. in DNA Fingerprinting: State of the Science. eds. Pena SDJ, Chakraborty R, Epplen JT, Jeffreys AJ. Birkhauser Verlag, Basel/Switzerland, 193-210.

Baum HJ, Fritz-Charles H and McKee R. (1990) A non-isotopic detection system with the sensitivity of P^{32}: Applications for paternity and forensic identifications. in Advances in Forensic Haemogenetics 3. eds: Polesky HF and Mayr WR. Springer-Verlag Berlin Heidelberg, 37-39.

Guisti A, Baird M, Pasquale S, Balazs I, Glassberg J (1986) Application of Deoxyribonucleic Acid (DNA) Polymorphisms to the analysis of DNA recovered from sperm. J Forensic Sciences 31:409-417.

Kanter E, Baird M, Shaler R, Balazs I (1986) Analysis of restriction fragment length polymorphisms in deoxyribonucleic acid (DNA) recovered from dried bloodstains. J Forensic Sciences 31:403-408.

Neuweiler J, Venturini J, Balazs I, Kornher S, Hintz D and Victor J. (1992) The use a chemiluminescent detection system for paternity and forensic testing. Verification of the reliability of the oligonucleotide-probes used for genetic analysis. in Advances in Forensic Haemogenetics 4. eds: Rittner Ch and Schneider PM. Springer-Verlag Berlin Heidelberg, 132-135.

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