FURTHER CHARACTERIZATION OF THE VNTR PROBE LH1 (D5S110) AND APPLICATIONS FOR DNA TYPING

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The single-locus probe LH1 (D5S110) originally described by Armour, J.P., et al, Genomics, Vol. 8, p.501, 1990 was evaluated for its effectiveness in DNA typing assays for human identification. A cloned, purified, 6-Kb DNA fragment containing sequences complementary to the D5S110 locus was labeled with ^{32}P-dCTP by the random primer method. LH1 appears to be one of the most sensitive single-locus probes observed, capable of detecting a DNA profile in 10 ng of Hae III restricted genomic DNA in a 24-hour autoradiographic exposure. An enzyme-linked oligonucleotide probe was constructed for use in a chemiluminescent hybridization and detection format. Nine repeats within the D5S110 cloned insert were sequenced, and a 30-base oligonucleotide was synthesized and labeled with alkaline phosphatase. The sensitivity and specificity of the alkaline phosphatase labeled oligonucleotide under the ACES 2.0 hybridization and detection conditions is comparable to that of the ^{32}P labeled LH1 probe. The enzyme-linked oligonucleotide probe was capable of detecting the correct banding pattern in 25 ng of Hae III restricted genomic DNA in a 3-hour exposure following a 3-hour ramp.

The LH1 probe was used in population studies at the University of North Texas Health Science Center at Fort Worth (UNTHSCFW, Texas, USA, and Genetic Design Incorporated (GDI, North Carolina, USA). A total of 3280 individuals were probed between the two labs (1144 African Americans, 1359 Caucasians, 777 Hispanics). The Hae III allelic distributions for the African American, Caucasian, and Hispanic ethnic groups were very similar between UNTHSCFW and GDI, with a linear correlation of 0.95, 0.97, and 0.91 respectively. The majority of alleles (African American 95%, Caucasian 99%, and Hispanic 98%) were broadly distributed between 640 and 10,000 base pairs. The observed heterozygosity at the D5S110 locus was also very similar in both population studies. The heterozygosity was greater than 95% for each of the three ethnic groups.

In 1800 cases examined between the two parentage testing laboratories, 19 maternal exclusions were observed for a maternal recombination rate of 0.0106. The combined maternal and paternal recombination rate observed at UNTHSCFW was 0.008. In cases were the alleged father had been excluded as the biological father, the probe LH1 failed to exclude the man approximately 4% of the time. The observed Exclusionary Power was greater than 95% for each of the three ethnic groups examined. Although the overall recombination rate is higher than some of the other single locus probes currently used for parentage testing in the United States, the broad distribution of alleles

combined with a very high exclusionary power would indicate that an analysis of the D5S110 locus would be extremely useful for the determination of paternity.

The utility of the LH1 probe in forensic casework has been examined at the Tarrant County Medical Examiners Office (Texas, USA). In several cases where a marginal amount of DNA was recovered from an evidentiary sample, a DNA profile was detectable with the LH1 probe. Other commonly used single locus probes such as YNH24, MS1, and PH30 occasionally failed to produce a profile either due to an insufficient or partially degraded sample. The sensitivity of the LH1 probe combined with the broad distribution of alleles between 640 and 10,000 base pairs makes it ideally suited for RFLP forensic casework. Forensic laboratories should strongly consider D5S110 as one of the primary loci analyzed in forensic samples.

CONCLUSION

The analysis of the D5S110 locus is extremely useful for human identification. The availability of the LH1 probe in both a radioactive and chemiluminescent format makes it one of the most powerful probes for DNA typing.