Characterization of DNA Probe pH30 (D4S139) and Preparation of Related Synthetic Oligonucleotides

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INTRODUCTION

An extremely sensitive, highly polymorphic variable number of tandem repeat (VNTR) human DNA clone, pH30, has been isolated and found to be useful for the typing of bio-forensic evidence (Milner, E., et al 1989).

CHARACTERIZATION AND GENE MAPPING

Gene mapping studies with multiple mouse/human somatic cell hybrids were used to localize pH30 to chromosome 4. This locus has been assigned the Human Gene Mapping Workshop number D4S139. Mapping was accomplished by linkage analysis performed on 18 large three generation and 6 two generation families (CEPH families) using 53 chromosome 4 markers. Linkage was found at a distance of 16 cM, and a LOD score greater than three, to L231 and C82, markers previously mapped near the telomere of the chromosome 4 long arm. Heterozygosity was observed at 89% in Caucasoids. Over 50 bands have been detected by pH30, the most common occurring at a frequency of 13 to 15% depending on the ethnic/racial composition of the population group under study (unpublished). Clone pH 30 is complimentary to the VNTR sequence as it detects restriction fragment variants in human DNA with over 20 different restriction enzymes. There is also a rare variant that has a Hae III site within the VNTR locus. Plasmid pH30 detects relatively large restriction fragments on Southern blots of human DNA, (avg. 9 Kb) when compared to most other VNTR probes (avg. 3 to 4 Kb). This characteristic makes ph30 highly useful forensic DNA Typing analysis.

ANALYSIS OF SEXUAL ASSAULT EVIDENCE

The DNA probe pH30 has been used on Southern blots of a series of non-probative sexual assault evidence swabs, stored frozen at -20°C for periods of up to one year prior to analysis. Southern blots were first probed with pH30, and then a Y chromosome specific probe. pH30 demonstrates a high level of sensitivity, being able to detect single copy

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VNTR sequences in approximately 10 nanograms of DNA. D4S139 VNTR bands were detected in 93% of cases (n = 43), and 85% of all swabs (n = 118) contained DNA. Of these 73% (n = 89) had readable VNTR patterns. Thirty percent of the female case derived swabs (n = 33) had Y chromosome specific DNA present, indicating that male DNA was present. Eighty-nine per-cent of oral swabs (n = 28) contained DNA, suggesting that buccal swabs, saliva or dried saliva stains may be a useful source of DNA.

DNA SEQUENCING

DNA sequence data, obtained from a pH30 1.5 Kb spontaneous deletion plasmid, suggests that D4S139 may be from a new family of VNTR loci. The DNA sequence of ten pH30 repeat units has been obtained. The repeat units, rather than being in tandem arrays, fall in groups of two or three, each group being separated by an eight base pair spacer. Each 32 base pair repeat unit was approximately 60% G/C, with an eight base pair relatively invariant core. The consensus sequence was 78% purines. The regions flanking the repeats were also unusual. They were greater than 80% A/T, with many runs of A or T, some as long as eight base pairs. Based on this data, oligonucleotides have been made corresponding to a consensus sequence, and are being used in hybridization studies to determine if sensitivity can be further improved.

CONCLUSION

Observed data on the VNTR probe pH30, suggests that it is highly useful for DNA typing of bio-forensic evidence. It exhibits a high level of polymorphism and heterozygosity and may be the most sensitive probe so far described.Reference.

REFERENCES

Milner, E.C.B. *et al* Isolation and mapping of a polymorphic DNA sequence pH30 on chromosome 4 [HGM provisional no. D4S139] (1989) Nucleic Acids Research, V. 17 No 10 4006.