PROJECT NO. 51/89 - Dr Anthony S. Weiss
Forensic Application of DNA Fingerprints: Limitations due to DNA Damage

This project used three methods of DNA identification: (1) DNA typing using single locus probes, (2) DNA fingerprinting with multi-locus probes, and (3) polymerase chain reaction (PCR) for sex determination. The hypothesis to test was whether DNA profiles remain constant over time, including the lifetime of an individual. This has relevance to the potential establishment of computer databases of DNA profiles of criminals as an aid towards identification and prosecution. Two families and unrelated controls have been examined to date and examined using the three criteria described. In all instances, there was no evidence for change in DNA profiles in these samples, even when cells from rapidly aging individuals were used. This provides evidence for the stability of DNA profiles over a person's lifetime.

DNA was extracted and purified from 13 human cell lines. These represented two families (A and B) - each having a progeroid child (family A: proband cell line 3513 and family B: proband cell line 3198). The DNA was then characterised by profiling and sex typing analysis.

The isolated DNA was digested with three restriction endonucleases: *Pst*I, *Hae*III and *Hin*fI, size separated by agarose gel electrophoresis, transferred to nylon membranes and hybridised with a single-locus probe YNH24 which recognises an informative region, D2S44, in the human genome.

The sizes of complementary DNA fragments binding to this probe were measured by comparing their relative mobilities to those of DNA size standards. The genotype of each individual at the D2S44 locus was determined, generating a DNA profile for each cell line. The genotype frequency of each cell line was calculated using databases containing the frequency of allele sizes identified within a sample population, at this locus, for each endonuclease. By extrapolating the databases to the level of the entire population, which is a significant assumption, the probability of a random individual having the same genotype as each cell line was determined. These values were typically less than 1/70. Due to the presence of segregating rare alleles within both families, this probability value was found to be quite low for some cell lines. As the most extreme example: 1/86 000 would have a *Pst*I-generated phenotype identical to that of cell line 3263. Such probabilities were generated even when using a relatively conservative approach for statistical analysis, although they did vary between databases used and were a factor of ten less, in most instances. Thus the level of individual identity of each cell line within the population was established.

The profiling technique also allowed pedigree determination as the alleles at such a locus segregate in a Mendelian fashion. It was found however, that while the alleged parental alleles in family A could be traced to the children and the genetic relationship between the cell lines established; in family B, pedigree could not be confirmed. The alleged parental cell lines (mother 3257 and father 3258) appeared to have an identical genotype. As the greatest probability of this occurring was estimated to be 1 in 600 people (1/600), this indicated that these cell lines had probably been derived from the same donor. The conclusion was confirmed by the fact that neither of these cell lines possessed an allele present in their alleged progeny, indicating that one of these cell liens was not a parent. Sex-typing using polymerase chain reaction (PCR) methodology was then performed in order to confirm the gender of the cell line donors under study. This confirmed the results established by probing, consolidating the conclusion that the

paternal cell line in family B was in fact a female - apparently identical to the maternal cell line. Re-sourcing of the parental donor tissues, helped to resolve this issue in work conducted after the CRC-funded period.

Further statistical extension of the CRC-funded results allowed the powers of paternal and maternal exclusion to be calculated for each family. Work carried out subsequent to the CRC-funded project period confirmed that pedigree could be fully assigned. Results from multilocus profiles are consistent with the conclusions reached using single-locus probes; thus banding patterns could be fully correlated with Mendelian inheritance and no new bands were observed. Data from skin fibroblast tissue samples were primarily taken from progeria cells aged substantially in culture. In addition, pulsed-field gel electrophoresis demonstrated that the size distribution of progeria DNA differed from that of control cells, and gamma irradiation appeared to have no effect upon limited hypervariable profiles. Taken together, the profiles were fully consistent with proposed pedigrees. These data are fully consistent with no alterations in the observed DNA profiles.

Although not funded by CRC, PCR human sex-typing procedure has formed the basis of a practical class experiment for the entire Science III undergraduate Biochemistry course (Weiss, 1991). This means that every student proceeding through this course (including Science-Law students) will receive some training in this aspect of DNA identification. A review has also been published for members of the legal profession describing aspects of DNA testing (Weiss, 1990).

References

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