AN INVESTIGATION OF VARIATION IN THE SIZING OF SHORT TANDEM REPEAT LOCI

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INTRODUCTION

Forensic identity testing has been transformed by the development of PCR-based systems to investigate polymorphic short tandem repeat (STR) loci, offering greatly increased sensitivity over DNA single locus probe testing while providing discrete allelic types (Lygo et al 1993). The use of automated DNA sequence apparatus to measure the size of STR alleles in DNA amplified with fluorescently labelled primers has increased the sensitivity of this system further. Alleles are sized with reference to an internal lane standard and types designated according to the number of repeat units present (DNA Commission of the International Society for Forensic Haemogenetics, 1992). Variation in the size measurement of alleles in an allelic ladder has been examined by Kimpton et al (1993); Lygo et al (1994) have extended this work to validate their forensic casework samples. The current study was performed to investigate, within our own laboratory, the reproducibility of STR sizes within and between gels. The same allelic types in different individuals were also examined to investigate whether variability was greater than when repeated measurements were made on the same sample.

MATERIALS AND METHODS

Samples: DNA, extracted using a 5% chelating resin, from blood samples of 90 mother-child pairs was used to examine within gel reproducibility. Each mother was run in a lane adjacent to her child and also separated from her child by half a gel. Five DNA extracts from individuals, selected to represent a range of allelic types for the STR alleles examined, were amplified both in bulk and stored frozen, and amplified freshly, for each analysis. Samples were analysed over ten gels, arranged in a Latin Square design to eliminate possible position bias, to examine the between gel reproducibility. To examine the variability of size measurement within STR alleles across a large number of unrelated people, amplified samples from 110 individuals were run over 15 gels.

PCR: PCR was carried out in a 5µl reaction volume with 2µl of DNA extract, 200mM dNTP, 0.25µM of primer for HUMVWA and HUMFES/FPS and 0.1U of Taq polymerase. PCR conditions were 94°C x 45s, 54°C x 60s, 72°C x 60s for 26 cycles

Automated fluorescent detection: 1µl of PCR product was mixed together with 2µl of formamide/dextran blue and 0.3µl of internal size standard GS2500. Samples were denatured at 95°C for 5 min before loading into a 36 well 6% polyacrylamide, 8M urea denaturing gel (Sequagel-6, National Diagnostics). Electrophoresis was carried out in an ABI 373 DNA Sequencer at 30W for 3 hours. Fragment sizes were analysed with 672 GeneScan software using a local Elder and Southern fit.

RESULTS

Within gel variation: Repeat measurements of STR alleles from the same person, or between common alleles in mother-child pairs, lay within a range of around 0.8 - 1bp for mother-child samples in adjacent lanes (n=87), and for samples separated by a half gel distance, whether mother-child pairs (n=87) or samples from the same person (n=87). The variation observed in HUMFES/FPS was similar to that in HUMVWA. However this excludes six HUMFES/FPS alleles which showed greater variation (up to 2.4 bp total range).

Between gel variation: The total size variation (minimum to maximum), observed in alleles from the five individuals and measured over ten gels, was also around 1bp, regardless of whether the amplified sample had been stored frozen for use, or prepared fresh for each gel. Eight outliers, around 1bp away from the bulk of the measurements, were observed with HUMFES/FPS. This phenomenon was associated with the observation of a split peak in the GS2500 internal 233bp size standard giving a shoulder at 232bp with a main peak at 233bp. The 233bp peak had been selected as the appropriate standard in these cases and this standard would have been used for the HUMFES/FPS allele sizing. This phenomenon was not seen with HUMVWA sizes which are smaller and do not involve the 233bp standard in their measurement.

Allele size variation in different individuals: Measurements of alleles (n=178) were made for HUMVWA and plotted in a histogram (Fig. 1). Seven alleles were observed with a maximum size variation of around 1.5bp, thus producing distinct allelic types. Windows from Lygo et al (1994) are shown superimposed over the data, illustrating the bunching of data towards the higher end of each window. Some data begins to fall outside the window towards the higher molecular weights, but generally the modes lie at a 4bp separation. Figure 2 shows the data for HUMFES/FPS (n=220 alleles). Seven allele types were observed but the spread of size measurements within any allele was greater (2-4bp). Superimposing appropriate windows, as described above, makes it clear that the modes of the data no longer lay 4bp apart and some appear to fall into the next allelic class.

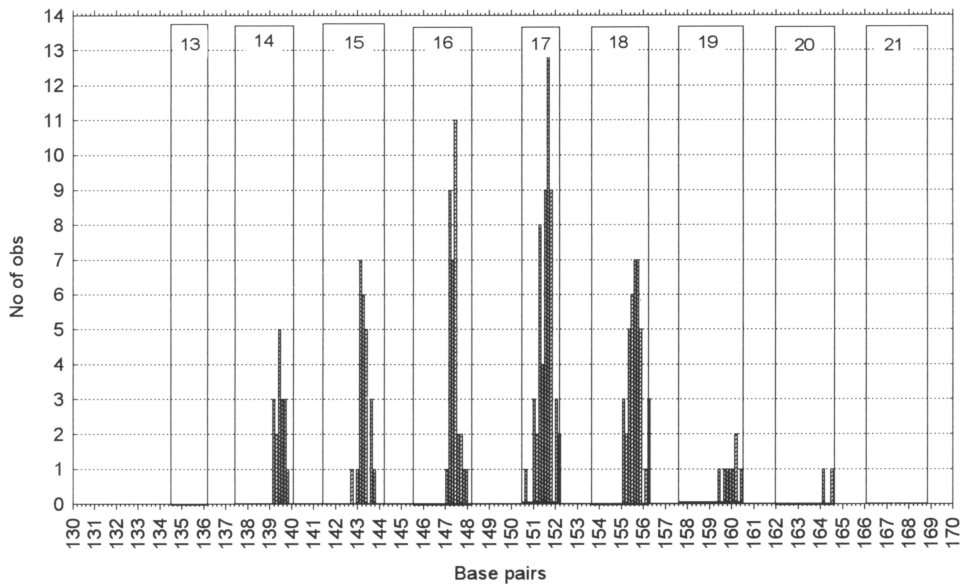

Figure 1: Histogram of molecular distribution of HUMVWA alleles with windows from Lygo et al (1994) superimposed

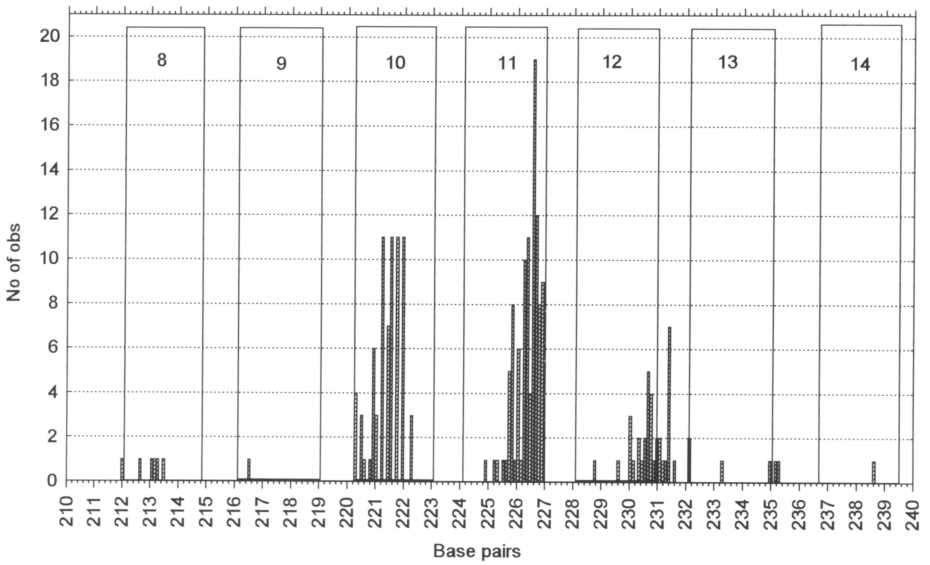

Figure 2: Histogram of molecular distribution of HUMFES/FPS alleles with windows from Lygo et al (1994) superimposed

DISCUSSION

Using allelic ladders Kimpton et al (1993) have reported within gel standard deviation for automated STR detection using the ABIs using an ABI 373 DNA Sequencer as between 0.07 and 0.12 bp, increasing to 0.31 bp between gels which is consistent with the 1bp maximum to minimum range we have observed between gels. We have not, however, observed a smaller variation within a gel, although possibly the variation in adjacent lanes is less. Lygo et al (1994) report a range of results from their analysis of allelic ladders and 24 case samples. Our samples mirror their results for HUMVWA, although the mode appears shifted by around 1bp. For HUMFES/FPS the modes of our data appear considerable shifted, with the spacing increasing towards the higher molecular weight. We are investigating whether this is associated with the observation of an occasional split peak on the GS2500 size standard. The designation of supposedly discrete allelic types thus depends on knowledge of the variation within any one system. Individual laboratories should fully validate their own windows before assigning type to avoid misclassification.

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