A UK CAUCASIAN DATABASE FOR THE TBQ7 (D10S28) LOCUS DERIVED FROM BLOOD SAMPLES SUBMITTED FOR PATERNITY ANALYSIS

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SUMMARY

The purpose of this study was to assess the potential value of the probe TBQ7, (locus D10S28), obtained from the Promega Corporation, for use in paternity testing.

The data was derived from DNA profiles obtained from blood samples submitted for parentage testing, and received by the FSS between April 1992 and January 1993. Only samples from Caucasian individuals were used in this study.

Raw data from DNA profiles derived from 374 unrelated Caucasian individuals is presented. The bands obtained ranged in size from about 700-7000bp. Twenty seven one banded individuals (OBI) were observed.

The raw data was smoothed using a 2.8% sliding window fit and a maximum frequency of approximately 0.08 observed. The heterozygosity was calculated to be 92.8%.

As the data was derived from individual DNA profiles obtained during parentage testing we were able to estimate the paternal and maternal mutation rates of 1.2% and 2.1% respectively.

METHODS

The DNA profiles were prepared using standard FSS protocols.

Hybridisation

The membranes used in this study had been previously hybridised with a series of single locus DNA probes, MS43a (D12S11), MS31 (D7S21), YNH24 (D2S44), and pMLJ14 (D14S13).

The membranes were incubated at 50oC for 20 minutes in hybridisation buffer containing 2.5ul TBQ7/ml buffer (Promega), and 0.2ul MW100/ml buffer (Cellmark).

Development and Detection of the DNA profiles

The membranes were dipped into Lumiphos (Cellmark Diagnostics) and the excess enzyme substrate removed by squeezing the membranes between two acetate sheet.

The autoradiographs were developed for 1-2 days at 37oC. Band sizes were determined by means of a video based image analysis system, (Foster and Freeman Ltd).

Results and Discussion

The chemiluminescent DNA probe TBQ7 (Promega Corporation) was found to detect a highly variable locus with the distribution of allele sizes ranging from 700-7000bp.

The heterozygosity was calculated as 92.8% which concurs with a more limited study of Bragg et al [2]. The heterozygosity of this probe was compared to published data on allele frequency distributions of four other VNTR loci [3] and the data presented in Table 1.

Two paternal and four maternal mutations were detected in this study giving a TBQ7 paternal mutation rate of 0.012 (2/167), and a maternal mutation rate of 0.021 (4/192). The paternal mutation rate for TBQ7 was compared with published data on paternal mutation rates for other VNTR loci [5] and the results are shown in Table 2.

PROBE	ALLELE SIZE RANGE (Kbp)	HETEROZYGOSITY %
TBQ7	0.7 - 7.0	92.8
MS1	1.2 - >20	96.1
MS31	1.8 - 15	91.8
MS43a	2.6 - >20	87.8
YNH24	1.6 - 9.2	88.3

TABLE 1 COMPARISON OF ALLELE SIZE RANGES AND PERCENTAGE HETEROZYGOSITY FOR FIVE SINGLE LOCUS PROBES [3]

PROBE	MATERNAL MEIOSES			PATERNAL MEIOSES		
	TOTAL	MUTATED	% MUTATED	TOTAL	MUTATED	% MUTATED
TBQ7	192	4	2.08	167	2	1.2
MS1	1116	45	4.03	591	36	4.16
MS31	1127	2	0.18	600	13	1.48
MS43a	1123	1	0.09	594	5	0.57
G3	1115	2	0.18	589	6	0.69
YNH24	471	1	0.21	344	0	0.00

TABLE 2 MEIOSES AND MUTATION RATES REPORTED AT 6 VNTR LOCI [1][5][4]

Sensitivity Experiments

The sensitivity of TBQ7 was tested with concentrations of 1ug to 50ng K562 genomic DNA. 150-200ng DNA was the limit of detection at about 1200bp. [6][3]

Hybridisation of K562 genomic DNA produced two alleles. Calculations based on 196 measurements showed the mean size of the two bands generated as 1748bp (sd=10) and 1178bp (sd = 8.5).

On occasion during this study the TBQ7 profiles generated showed more than two bands. Analysis of 20 such profiles that showed more than two bands showed that the difference in molecular size between the upper and 'true' lower band was 203bp (range 179-229).

Statistical analysis

The raw data was generated by analysis of the DNA profiles of 374 unrelated individuals, ie mothers and fathers of unrelated family trios. Binning the data into 100bp bins allowed the comparison of the TBQ7 data and that generated for MS1, MS31, MS43 and YNH24 [1].

A 2.8% sliding window fit was applied to the raw data shown in Figure 2. This involved calculating the frequency for each allele at 5 bp intervals based on a +/- 2.8% bin. The resulting plot is shown in Figure 3.

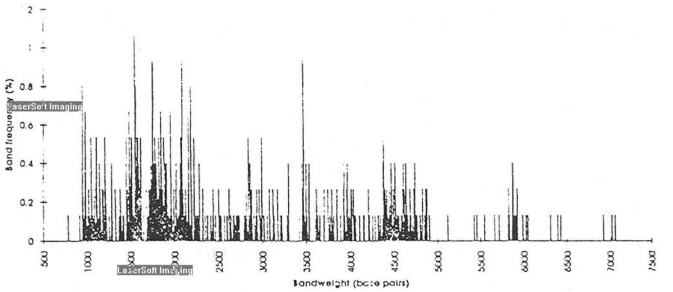

Figure 2: Raw data plot for FSS RC1 TBQ7 paternity data calculated based on a 5 base pair 'bin' for 374 profiles including 27 one-banded individuals.

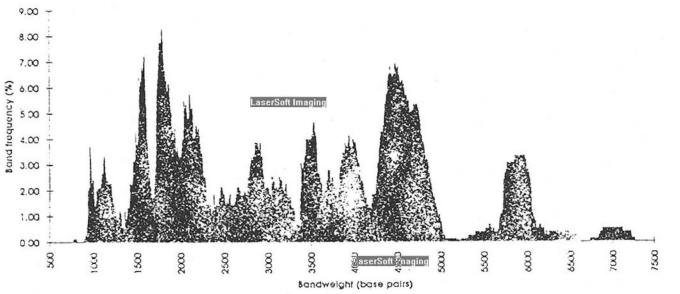

Figure 3: 2.8% sliding plot for FSS RC1 TBQ7 paternity data calculated at a 5 base pair interval for 374 profiles including 27 one-banded individuals.

A statistical analysis of the TBQ7 data to assess the practical effects of between probe dependence was conducted along similar lines to the experiments described in Evett et al [7]. TBQ7 data was compared to the data generated by the probes YNH24, MS31, MS43a, and pMLJ14. Although the value of the exercise may be limited by the relatively small database there was no evidence to suggest that the assumption of independence is invalid for any of these probe combinations.

Conclusions

The wide allele distribution (700-7000bp), the high percentage heterozygosity (93.5%) and the relatively low percentage paternal mutation rate (1.2%), indicated that the VNTR probe TBQ7 (Promega Corporation) is an extremely informative probe for use in paternity testing.

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