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The Development of DNA Profiling Database in an HAE III Based RFLP System for Chinese, Malays, and Indians in Singapore

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ABSTRACT: Deoxyribonucleic acid (DNA) restriction fragment length polymorphism (RFLP) profiles were obtained for blood specimens from the three population groups—Chinese, Malays and Indians—in Singapore. The population databases were collected from Hae III digested high molecular weight DNA hybridized with four variable number of tandem repeats (VNTR) loci - D2S44, D10S28, D4S139 and D1S7. The data were analyzed statistically using the fixed bin system. Comparison of ratio of bin frequencies of these population data with published data on whites, blacks, and hispanics shows that the alleleic distribution at these loci is not seriously different among the six groups. This has important implications to the statistical significance of forensic DNA applications.

KEYWORDS: pathology and biology, Deoxyribonucleic acid (DNA), restriction fragment length polymorphism (RFLP), variable number of tandem repeats (VNTR), allele frequency, paternity index, Chinese, Malays, Indians, binning system, population database

The genetic characterization of evidential materials by restriction fragment length polymorphism (RFLP) has been implemented in a number of advanced forensic laboratories throughout the world [*1–3*]. This technology also provides a powerful means of determining parentage and relatedness [*4,5*]. Each RFLP system is characterized by a unique combination of one restriction enzyme with a number of different probes. The FBI [*2*] and the Royal Canadian Mounted Police [*3*] use the enzyme Hae III and single locus probes such as D2S44, D10S28, D17S79 and D4S139 while the Home Office Forensic Science Service, England [*1*] uses the enzyme Hinf I and a number of multi- and single-locus probes. Irrespective of the enzyme and probes used, the population data were collected mainly for whites, blacks and hispanics with few data on Asians. Singapore is a multiethnic country, the three main population groups are Chinese, Malays, and Indians. This paper describes the DNA profiling database developed for the Chinese, Malays,

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and Indians population in Singapore based on Hae III and four variable number of tandem repeats (VNTR) loci - D2S44, D10S28, D4S139 and D1S7.

Materials

Whole blood samples from unrelated Chinese, Malays, and Indians were obtained from the Singapore Blood Transfusion Service. Hae III, D2S44, D10S28 and human cell line K562 were obtained from Promega Corp, Madison, WI. D1S7 was purchased from Cellmark Diagnostics, Abingdon, Oxfordshire, UK. D4S139 and DNA size marker (ranging in size from 526 to 22 621 base pairs) were purchased from Bethesda Research Laboratories, MD. All chemicals and reagents used were of analytical grade.

Methods

Thawed frozen whole blood samples and K562 cells were extracted twice with phenol/chloroform/isoamyl alcohol after proteinase K digestion. DNA was precipitated by absolute ethanol. The DNA was solubilized in TE buffer (10 mM Tris/HCl, 0.1 mM EDTA, pH 8.0) and dialyzed against TN buffer (10 mM Tris/HCl, 10 mM NaCl, pH 7.6). Quantitations were achieved spectrofluorometrically with Hoechst dye in a Hoeffer DNA fluorometer TKO100 or mini-gel electrophoresis with standards of known quantity.

After quantitation, 800 ng of DNA was restricted overnight with ten-fold excess of the enzyme Hae III. Completeness of digestion was assessed by mini-gel electrophoresis using less than 1% of the digest. The remaining Hae III digested DNA was subjected to electrophoresis in a 0.8% agarose gel (BRL Ultrapure Agarose). Each gel contained three lanes of size marker flanking all DNA samples and two lanes of Hae III digested K562 DNA as control samples. The gels were 20 cm × 25 cm and were run without ethidium bromide at 45 V for 16 h in 1 × TBE buffer (130 mM Tris base, 75 mM boric acid, 2.5 mM EDTA, pH 8.8). The DNA were alkaline blotted onto nylon membranes (Hybond N+) followed by heat baking at 80°C for 30 min.

Probes were labeled by random priming (Promega Prime-a-gene-kit) and cleaned with a Sephadex G-50 column. Hybridization according to the method of Church and Gilbert was done at 65°C overnight followed by high stringency washes (0.1 × SSC/0.1% SDS). Autoradiography was carried out at −80°C using Kodak XAR 5 film with intensifying screens for periods up to three days.

Each autoradiograph was analyzed by three operators. The distance traveled from the origin by each band of the size ladders and the profiles was manually measured and entered into a computer. The readings were normalized and fragment sizes were calculated using a cubic spline interpolation program (MathCAD). Population frequencies were then generated based on the binning method of Budowle et al. [2] using the BRL DNA Analysis Size Marker.

Results and Discussion

Singapore is a multiethnic country with three main population groups—Chinese, Malays, and Indians. The DNA RFLP allele frequency databases have been created for these three main groups. So far over 200 Chinese, 200 Indian, and 200 Malay individuals have been analyzed with the probes D2S44, D10S28, D4S139 and D1S7 by the method described previously. In order to estimate the RFLP allele frequencies, the fixed bin method of Budowle et al. [2] was adopted. A total of 30 bins were arbitrarily constructed based on the BRL DNA analysis marker that was incorporated in every autoradiograph (three lanes per autoradiograph). Each autoradiograph contains two K562 cell line profiles that act as an internal quality control for size measurements and assessment of standard

TABLE 1—*Bin frequency distribution table.*

Bin No.	Range, Base Pair	D2S44 (YNH24)			D10S28 (TBQ7)			D4S139 (PH30)			D1S7 (MS1)		
		Chn	Ind	Mal	Chn	Ind	Mal	Chn	Ind	Mal	Chn	Ind	Mal
1	0–526	0	0	0	0	0	0	0	0	0	0	0	0
2	527–653	0	0	0	0	0	.002	0	0	0	0	0	0
3	654–784	0	0	0	.009	.022	.002	0	0	0	0	0	0
4	785–910	.043	0	.047	.024	.014	.020	0	0	0	0	0	0
5	911–993	.010	.015	.012	.073	.031	.048	0	0	0	0	0	0
6	994–1176	.013	.056	.052	.104	.048	.087	0	0	0	0	.002	0
7	1177–1287	.025	.046	.037	.035	.038	.022	0	0	0	.002	.002	.005
8	1288–1431	.070	.105	.097	.066	.046	.035	0	0	0	.005	.005	.011
9	1432–1588	.148	.105	.201	.080	.079	.050	0	.002	0	.010	.007	.016
10	1589–1672	.110	.093	.082	.033	.036	.055	0	0	0	.002	.002	.005
11	1673–1861	.185	.176	.112	.045	.087	.048	0	0	0	.020	.005	.014
12	1862–2015	.088	.073	.052	.035	.084	.055	0	0	0	.022	.012	.011
13	2016–2213	.065	.049	.055	.061	.087	.066	0	.012	.005	.034	.025	.025
14	2214–2432	.080	.054	.042	.045	.041	.059	.002	.017	.002	.027	.045	.018
15	2433–2650	.073	.061	.077	.073	.043	.050	.002	0	.005	.039	.042	.025
16	2651–2876	.043	.041	.035	.099	.079	.096	.012	.005	.018	.017	.035	.029
17	2877–3101	.030	.054	.042	.068	.038	.124	.010	.007	.009	.032	.047	.038
18	3102–3397	.010	.029	.015	.033	.065	.059	.022	.024	.032	.059	.050	.034
19	3398–3812	.005	.029	.020	.038	.048	.048	.047	.049	.032	.093	.075	.074
20	3813–4333	.003	.007	.010	.057	.046	.028	.077	.034	.050	.098	.104	.090
21	4334–4716	0	0	.005	.014	.010	.015	.074	.039	.046	.076	.077	.068
22	4717–5415	0	0	.002	.007	.031	.017	.178	.109	.144	.100	.077	.099
23	5416–5861	0	0	0	0	.007	0	.111	.075	.126	.034	.040	.074
24	5862–6442	0	.002	.002	0	.005	.009	.104	.146	.096	.073	.042	.088
25	6443–7421	.003	0	0	0	0	0	.196	.175	.206	.061	.097	.101
26	7422–8271	0	.002	0	0	.005	.004	.077	.097	.096	.032	.067	.041
27	8272–9416	0	.002	0	0	.007	0	.050	.107	.085	.066	.040	.036
28	9417–11919	0	0	0	0	.002	0	.032	.078	.025	.071	.055	.059
29	11920–15004	0	0	0	0	0	0	.005	.019	.018	.024	.035	.034
30	15005–22621	0	0	0	0	0	0	0	.005	.002	.005	.010	.007
	Number of persons	200	205	201	212	208	229	202	206	218	205	201	222

deviation for our electrophoretic system. The standard deviations for all four loci from all database autoradiographs range from 1.02 to 1.36 with an average of 1.14. The standard deviations are almost twice those reported by Budowle et al. [2], but are comparable to other reported precision data [6] on VNTR analysis.

Table 1 lists the binned frequencies of the various VNTR loci for Chinese, Malays, and Indians. Table 1 reveals interesting difference at the bin level between the races. Bin 6 for D2S44 reveals a four-fold difference for the Chinese compared with the Malays and Indians. This is seen again at bin 11 for D1S7, where the Chinese reveal a four-fold higher frequency compared to the Indians. In the data reported by Budowle et al. [2] for whites, blacks, and Hispanics at six loci, a three-fold difference between the whites and blacks is seen at the bin bracketed by 1078-1196 bp at the locus D2S44. Weir [7] examined the population data (three racial groups from three geographic areas) of the FBI and concluded that there was evidence for frequency differences at the bin level between geographic samples of the same races and between races. Frequency variation at the bin level for different races should be expected and is consistent with frequency variation between races for alleles of traditional blood markers and protein systems.

While bin frequencies may differ between races, the distribution of alleles at any locus shows a consistent pattern (Figs. 1–4). At D2S44, the bin frequencies appear to be similar for the three races from bin four to bin 27 even though the Malays bin frequencies peak (0.201) at a lower fragment size (bin nine, 1432-1588 bp) than the Chinese and Indians (0.185, 0.176 respectively, bin 11, 1673 - 1861 bp). The distribution of the bin frequencies at locus D10S28 (Fig. 2) has a similar but wider spread than D2S44 and consequently lower maximum frequencies that D2S44. The fragments obtained at locus D4S139 (Fig. 3) for these three races were of the larger fragment sizes ranging from bin nine to bin 30. All three races have the bin frequencies peaks at bin 25 (6443-7421 bp). The allelic distribution at locus D1S7 (Fig. 4) was also of higher fragment sizes like D4S139, but spread wider, with the peak at bin 20 (3813-4333 bp). The consistent pattern for distribution of alleles for the four VNTR for the three population groups suggest that the three races may not be seriously different at the four VNTR.

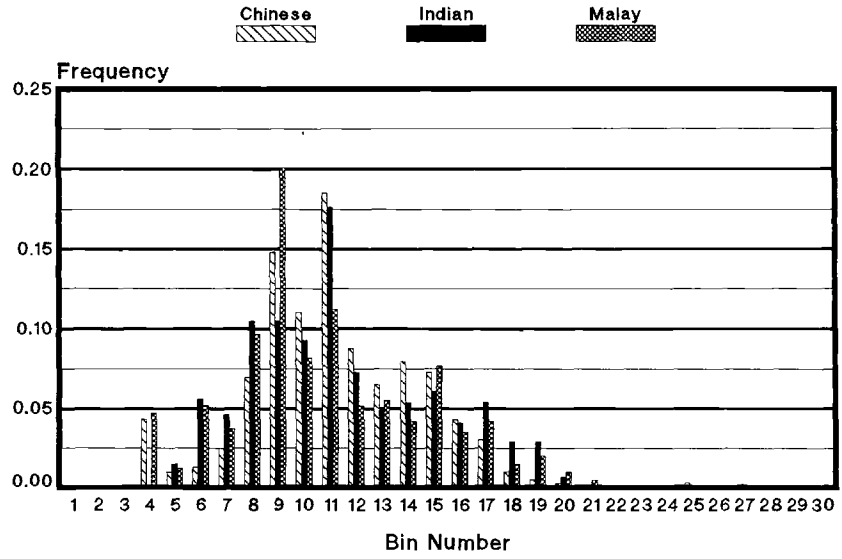

FIG. 1—*Frequency Distribution Chart HaeIII digested - D2S44.*

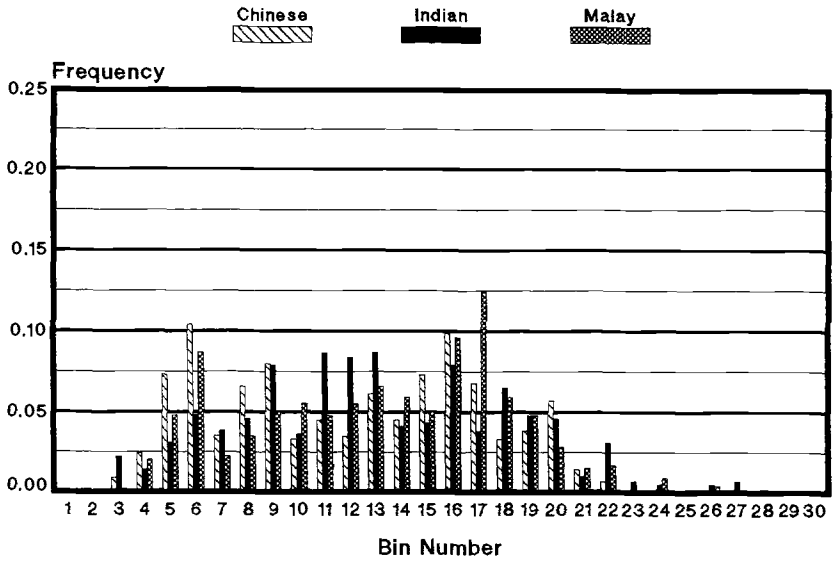

FIG. 2—*Frequency Distribution Chart HaeIII digested - D10S28.*

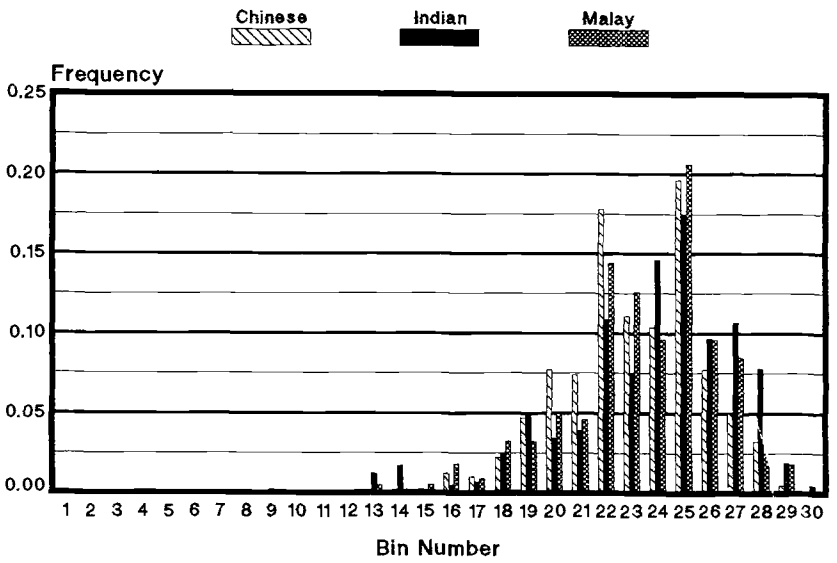

FIG. 3—*Frequency Distribution Chart HaeIII digested - D4S139.*

These figures show that all the loci are highly polymorphic. Two of the loci, D2S44 and D10S28, cover the lower fragment sizes ranging from 700 bp to 8000 bp, while the other two loci cover the larger fragment sizes ranging from 1000 bp to 20 000 bp. Combination of these four VNTR loci thus cover almost the whole range of RFLP fragments seen under the experimental conditions.

Table 2 shows some features of allele frequency population databases for Chinese, Malays, and Indians respectively. The most common combined genotype frequency ob-

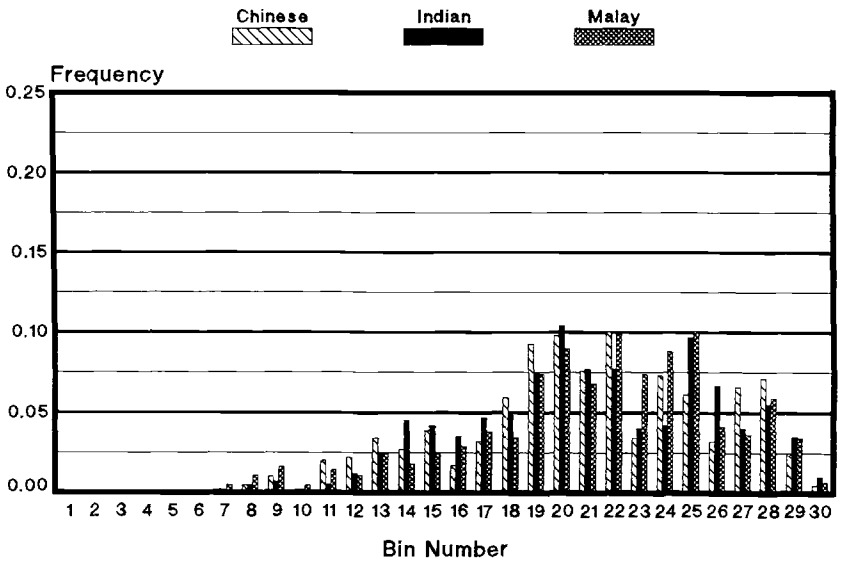

FIG. 4—*Frequency Distribution Chart HaeIII digested - D1S7.*

served was 8×10^{-3} (or 1 in 125) for the Malay population. Single band profiles were calculated as 2p, p being the frequency of the single band. This is a common and conservative approach adopted for forensic applications [*1*,*2*].

DNA RFLP has received widespread attention and discussion because of its forensic application. A match of two DNA profiles between DNA recovered from biological crime scene specimens and a suspect is a means to link the suspect to the crime. The probability of another person, selected at random, having the same DNA profiles is usually calculated by multiplying the allele frequencies of all the VNTR loci tested from a reference database. Although this approach has been subjected to some criticism [*8*,*9*], the binning method has been accepted [*3*,*10*] as an operational means to obtain conservative estimates of the probability of DNA profiles. This approach is supported by Weir [*7*] who demonstrated the independence of VNTR alleles defined as fixed bins. His results from an analysis of six VNTR loci for three races from three geographic locations also justified the multiplication rule currently used to obtain frequencies of multi-loci profiles.

In order to compare the difference of bin frequencies between our data and those reported for whites, blacks, and hispanics, we propose that a simple and quick method is to calculate the ratios of bin frequencies for each corresponding bin. This was done by rebinning our data and those of blacks and composite hispanics to the rebinned ranges of whites [*11*]. Table 3 shows the rebinned frequency distribution for locus D4S139. In our data, any bin with a frequency less than 0.01 was given a minimum bin frequency of 0.01 as recommended by Evett et al. [*12*]. The lower frequency of each corresponding pair was used as the denominator to provide a positive ratio. This will give a minimum ratio of 1, if the bin frequencies of the two corresponding bins are equal. At each bin for each locus, the bin frequency of one population group was compared to each of the other five population groups, giving 5 ratio values. Table 4 summaries the ratio values calculated for the four VNTR loci. The first value for D2S44 shows that 69.84% of ratio values obtained are in the range of 1 to 1.9. The results show that more than 70% of the ratios are less than two and more than 96% of the ratios are less than five. Only D4S139 shows a bin frequency ratio of more than ten with an extremely low percentage

TABLE 2—*Features of allele frequency population databases for Chinese, Malays, and Indians.*

Race	Locus	No. of persons	Heterozygote, %	Most common bin frequency	Most common genotype frequency[a]	Combined genotype frequency[b]
Chinese	D2S44	200	91.5	0.185	0.370	
	D10S28	212	94.8	0.104	0.208	
	D4S139	202	91.6	0.196	0.392	
	D1S7	205	94.1	0.100	0.200	6×10^{-3} (1 in 166)
Malays	D2S44	201	93.5	0.201	0.402	
	D10S28	229	96.1	0.124	0.248	
	D4S139	218	91.7	0.206	0.412	
	D1S7	222	91.4	0.101	0.202	8×10^{-3} (1 in 125)
Indians	D2S44	205	94.6	0.176	0.352	
	D10S28	208	94.7	0.087	0.174	
	D4S139	206	88.3	0.175	0.350	
	D1S7	201	91.5	0.104	0.208	4×10^{-3} (1 in 250)

[a]Assuming Hardy-Weinberg equilibrium, the most common homozygous (2p) or heterozygous (2pq) genotype was derived.
[b]Product of the most common genotype frequencies of each locus.

TABLE 3—*Rebinned frequency distribution table for D4S139.*

Frequency range	Cau	Blk	His	Mal	Ind	Chi
0–2522	.004	.069	.012	.011	.032	.002
2523–2692	.010	.016	.012	.002	.012	.002
2693–3033	.006	.016	.018	.021	.012	.020
3034–3329	.014	.012	.013	.032	.012	.017
3330–3674	.031	.021	.024	.025	.046	.037
3675–3979	.023	.052	.043	.028	.017	.032
3980–4323	.040	.063	.045	.032	.032	.059
4324–4821	.047	.077	.061	.069	.049	.099
4822–5219	.054	.064	.063	.083	.053	.109
5220–5685	.072	.066	.104	.135	.097	.124
5686–6368	.108	.081	.137	.106	.146	.116
6369–7241	.191	.084	.167	.188	.172	.186
7242–8452	.131	.103	.175	.149	.153	.121
8453–10093	.095	.109	.130	.083	.121	.050
10094–11368	.036	.077	.040	.014	.029	.020
11369–12829	.035	.029	.032	.009	.007	.002
12830–22621	.102	.061	.071	.014	.022	.002

TABLE 4—*Percent of ratios of bin frequencies among Chinese, Malays, Indians, whites, blacks, and hispanics.*

Ratio of bin frequencies	Percent of Ratio			
	D2S44	D10S28	D4S139	D1S7
1–1.9	69.84	71.88	77.25	74.36
2–2.9	12.38	19.42	13.73	12.56
3–3.9	11.43	4.93	4.31	12.05
4–4.9	4.44	2.61	0.78	0.77
5–5.9	0.32	1.16	1.18	0.26
6–6.9	0.32	0	1.57	0
7–7.9	1.27	0	0.78	0
8–8.9	0	0	0	0
9–9.9	0	0	0	0
10–11.9	0	0	0.39	0
12 and above	0	0	0	0

of 0.39%. This is because of the low frequencies obtained at bin 17 (12830 to 22621 bp as shown in Table 3) for the Chinese, Malays, and Indians.

To analyze the significance of these ratios, we calculated the ratios of bin frequencies between whites and Indians as they were considered to be in the same race group [*13*]. Table 5 shows the percent of ratios of bin frequencies between whites and Indians for the four VNTR loci. Comparing the data in Table 4 against the data in Table 5, a similar pattern was observed, that is, over 70% of the ratios are less than two. These results suggest that the distribution of VNTR alleles at these loci is not forensically significant [*9*] among the six groups. This means that the rarity of composite DNA profiles would not change regardless of the reference database used, which has great significance for forensic applications. Our results are consistent with Chakraborty and Kidd [*9*] who concluded that even if different databases were used, representing statistically significant allele frequency differences, the inference about the rarity of occurrence of each multi-loci DNA profile remained virtually unaltered and in occasions of the unavailability of population-specific allele frequencies, valid estimates of multi-loci genotype probabilities could be obtained from alleles frequencies for the pooled population. In essence, this is

TABLE 5—*Percent of ratios of bin frequencies between whites and Indians.*

Ratio of bin frequencies	Percent of Ratio			
	D2S44	D10S28	D4S139	D1S7
1–1.9	71.43	69.57	82.62	84.62
2–2.9	23.81	21.74	0	15.38
3–3.9	4.76	4.35	11.76	0
4–4.9	0	4.34	5.88	0
5–5.9	0	0	0	0
6–6.9	0	0	0	0
7–7.9	0	0	0	0
8–8.9	0	0	0	0
9–9.9	0	0	0	0
10–11.9	0	0	0	0
12 and above	0	0	0	0

a similar conclusion [*12,14*] that a reference database of any ethnic group could be used to obtain a reliable estimate in occasions where the allele frequences of a particular ethnic group is unavailable provided a 1% minimum default frequency is used in the calculation.

To analyze the relative rarity of our DNA profiles, we used our database and compared every individual against all the others in the database and found that the probability of finding a match for a single locus DNA profile was 0.223, 0.083, 0.26 and 0.051 for D2S44, D10S28, D4S139, and D1S7 respectively. This is consistent with the distribution pattern discussed earlier (Figs 1-4). However, there was no match among the 600 profiles for a 4-loci genotype.

To evaluate these four VNTR loci for paternity analysis, the average power of exclusion and mean paternity index were calculated according to the equation of Brenner and Morris [*15*]. The data are listed in Table 6 and 7 respectively. The power of exclusion

TABLE 6—*Average power of exclusion.*

Probe	Chinese	Malays	Indians
D2S44	82.62%	86.80%	89.07%
D10S28	89.43%	92.02%	89.23%
D4S139	82.79%	83.12%	76.18%
D1S7	88.06%	82.50%	83.45%
Cumulative PE	99.96%	99.97%	99.95%

*Calculated using equation of Brenner and Morris [*15*].
Average Power of Exclusion = $h^2(1\text{-}2hH^2)$.
h = heterozygosity, H = homozygosity.

TABLE 7—*Mean paternity index.*

Probe	Chinese	Malays	Indians
D2S44	5.88	7.73	9.32
D10S28	9.64	12.72	9.45
D4S139	5.94	6.06	4.29
D1S7	8.54	5.84	6.18
Cumulative PI	2877	3479	2335
Probability of paternity	99.97%	99.97%	99.96%

*Calculated using equation of Benner and Morris [*15*].
Mean paternity index = 1/2H.

range from 76% for D4S139 (Indians) to 92% for D10S28 (Malays). These figures agree with the power of exclusion for laboratories using Hae III for paternity analysis [*16*]. The minimum cumulative power of exclusion and probability of paternity for the four VNTR loci for our population are 99.95% (Indians) and 99.96% (Indians), respectively.

Conclusion

The Hae III RFLP system with four VNTR loci - D2S44, D10S28, D4S139 and D1S7 displays a high degree of polymorphism over the entire RFLP range and a high degree of discrimination with a minimum combined genotype frequency 8×10^{-3} (that is, 1 in 125). These loci also exhibit a minimum cumulative probability of paternity of 99.96%. This set of four VNTR loci is, therefore, suitable and sufficient for forensic applications. No match was found among the 600 DNA profiles in our database for a 4-loci genotype. Comparison of the ratios of bin frequencies among the Chinese, Indians, Malays, whites, blacks, and Hispanics shows that difference of allelic distributions at these loci is not forensically significant. Consequently, the rarity of occurrence of each multi-loci DNA profile remains virtually unaltered and in occasions of unavailability of population specific alleles frequencies, valid estimates of multi-loci genotype probability can be obtained from alleles frequencies for the pooled population.

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