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Announcement of Population Data

Allele frequencies of six miniSTR loci of three ethnic populations in Singapore

R.Y.Y. Yong [a,*], L.S.H. Gan [a], M.D. Coble [b], E.P.H. Yap [a]

[a] *Defence Medical and Environmental Research Institute, DSO National Laboratories, 27 Medical Drive, Singapore 117510, Singapore*
[b] *National Institute of Standards and Technology, Biotechnology Division, 100 Bureau Drive, Mail Stop 8311, Gaitherburg, MD 20899, USA*

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Abstract

MiniSTR loci has demonstrated to be an effective approach to recover genetic information from degraded sample, due to the improved PCR efficiency of their reduced PCR product sizes. This study investigated the allele frequency of six miniSTR loci, D1S1677, D2S441, D4S2364, D10S1248, D14S1434 and D22S1045, in three Singapore populations. All loci showed a moderate degree of polymorphism with observed heterozygosity >0.6 for all three populations. The allele frequencies, forensic parameters and heterozygosity comparison with other CODIS STR in similar populations are presented.

Keywords: MiniSTR; Allele frequencies; Population data; Singapore

Population: Venous blood was obtained from randomly selected Singapore Armed Forces personnel comprising 185 Chinese, 182 Malay and 178 Indian individuals. Samples were anonymised and the collection procedure approved by DMERI Research Ethics Committee. Singapore is a small multiracial state with a total resident population of 3.5 million, primarily comprising of three recognized ethnic groups; 76% Chinese, 14% Malay and 9% Indian [1]. While the Chinese and Indian communities mostly comprise migrants from Southern China and India, respectively in the last 200 years, the Malay has been resident in the region (the Malayan Peninsular) for a much longer time.

Extraction: Genomic DNA was extracted from 200 μl of whole blood using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). DNA quantitised by spectrophotometry.

Marker panel and PCR: Two multiplexes (mini01 and mini02) were performed with the same primer sets as in Coble and Butler [2]. Changes were made to the fluorescent dye label to accommodate subsequent PCR fragment analysis detection in the automated sequencer MegaBACE1000. The forward primers of D10S1248 and D4S2364 were labelled with 6FAM, D14S1434 and D2S441 with HEX, and D22S1045 and D1S1677 with TET. Each PCR multiplex was performed in a total volume of 10 μl containing 1 ng genomic DNA, 1× Amplitaq Gold buffer, 0.5 U Amplitaq Gold polymerase (Applied Biosystems, Foster City, USA), 1.5 mM $MgCl_2$, 200 μM of each deoxyribonucleotide triphosphate, and similar primer concentration as published [2]. Amplification were done in GeneAmp 9700 (Applied Biosystems, Foster City, USA) with slight modification in PCR condition. Pre-PCR denaturation was carried out at 95 °C for 10 min, followed by 30 cycles of 94 °C for 20 s, 55 °C for 20 s, 72 °C for 20 s, and a final extension of 60 °C for 45 min.

Genotyping: PCR products were separated on an automated capillary electrophoresis sequencer (MegaBACE1000, Molecular Dynamics, Sunnyvale, USA). Multiplex PCR product was diluted 15 times. One microlitre of the diluted product was mixed with 4 μl of loading solution (0.1% Tween 20) containing 0.125 μl of ET400-R internal size standard. Samples were denatured at 95 °C for 2 min, snap-cold on ice, and injected for 80 s at 3 kV, electrophoresis run at 10 kV for 75 min at 44 °C. Genotypes were called with Fragment Profiler (Version 1.2).

* Corresponding author. Tel.: +65 64857252; fax: +65 64857262.
E-mail address: ritayong@dso.org.sg (R.Y.Y. Yong).

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Table 1
Allele frequency distributions of six miniSTR loci in three Singapore populations

Miniplex 01

D10S1248				D14S1434				D22S1045			
Allele	Chinese ($N = 185$)	Malay ($N = 182$)	Indian ($N = 178$)	Allele	Chinese ($N = 185$)	Malay ($N = 182$)	Indian ($N = 178$)	Allele	Chinese ($N = 185$)	Malay ($N = 182$)	Indian ($N = 178$)
10	0.0027	–	–	14	0.0730	0.0824	0.171	7	–	–	0.006
11	0.0027	–	–	15	0.1676	0.1154	0.118	8	0.1784	0.1703	0.264
12	–	–	0.008	16	0.0243	0.0165	0.037	9	–	–	0.003
13	0.0784	0.0522	0.020	17	0.2838	0.2857	0.270	10	–	0.0055	0.003
14	0.3595	0.3104	0.191	17.3	0.0027	–	–	11	0.0324	0.0522	0.093
15	0.2243	0.2363	0.253	18	0.4189	0.4588	0.371	12	0.3054	0.3819	0.396
16	0.2324	0.2473	0.272	19	0.0189	0.0330	0.022	13	0.2297	0.1621	0.160
17	0.0838	0.1099	0.197	20	0.0108	0.0082	0.008	14	0.2324	0.2088	0.070
18	0.0162	0.0412	0.056	21	–	–	0.003	15	0.0189	0.0137	0.003
19	–	0.0027	0.003					16	0.0027	0.0055	0.003

Miniplex 02

D1S1677				D2S441				D4S2364			
Allele	Chinese ($N = 185$)	Malay ($N = 182$)	Indian ($N = 178$)	Allele	Chinese ($N = 185$)	Malay ($N = 182$)	Indian ($N = 178$)	Allele	Chinese ($N = 185$)	Malay ($N = 182$)	Indian ($N = 178$)
9	0.0027	0.0027	–	8	–	–	0.003	8	0.0054	–	–
10	–	0.0027	0.006	9	–	–	–	9	0.1784	0.2060	0.171
11	0.0162	0.0247	0.051	10	0.2270	0.2308	0.343	10	0.4243	0.3571	0.494
12	0.1054	0.1319	0.124	11	0.3757	0.2720	0.382	11	0.3811	0.4341	0.326
13	0.4892	0.4890	0.427	11.3	0.0622	0.1896	0.062	12	0.0108	0.0027	0.008
13.1	–	0.0027	–	12	0.2027	0.1099	0.065				
14	0.3000	0.3049	0.295	12.3	–	0.0055	-				
15	0.0757	0.0357	0.093	13	0.0135	0.0110	0.017				
16	0.0108	0.0055	0.003	14	0.1135	0.1676	0.110				
17	–	–	0.003	15	0.0054	0.0137	0.014				
				16	–	–	0.006				

Table 2
Forensic parameters of six miniSTR loci in three Singapore populations

	D10S1248			D14S1434			D22S1045		
	Chinese ($N = 185$)	Malay ($N = 182$)	Indian ($N = 178$)	Chinese ($N = 185$)	Malay ($N = 182$)	Indian ($N = 178$)	Chinese ($N = 185$)	Malay ($N = 182$)	Indian ($N = 178$)
Miniplex01									
Observed heterozygosity	0.795	0.720	0.736	0.676	0.714	0.747	0.816	0.742	0.719
Power of discrimination	0.895	0.906	0.919	0.870	0.855	0.888	0.899	0.900	0.882
Polymorphism information content	0.714	0.734	0.748	0.663	0.639	0.705	0.728	0.716	0.694
Power of exclusion	0.589	0.460	0.486	0.392	0.451	0.505	0.629	0.496	0.458
Typical paternity index	2.434	1.784	1.894	1.542	1.750	1.978	2.721	1.936	1.780
HWE p-values	0.622	0.050	0.148	0.370	0.944	0.126	0.824	0.477	0.148
	D1S1677			D2S441			D4S2364		
	Chinese ($N = 185$)	Malay ($N = 182$)	Indian ($N = 178$)	Chinese ($N = 185$)	Malay ($N = 182$)	Indian ($N = 178$)	Chinese ($N = 185$)	Malay ($N = 182$)	Indian ($N = 178$)
Miniplex02									
Observed heterozygosity	0.643	0.632	0.764	0.719	0.758	0.697	0.600	0.610	0.640
Power of discrimination	0.826	0.825	0.862	0.900	0.926	0.872	0.807	0.802	0.775
Polymorphism information content	0.599	0.591	0.658	0.711	0.765	0.671	0.570	0.567	0.547
Power of exclusion	0.346	0.331	0.534	0.458	0.524	0.423	0.291	0.303	0.342
Typical paternity index	1.402	1.358	2.119	1.779	2.068	1.648	1.250	1.282	1.391
HWE p-values	0.758	0.919	0.461	0.453	0.143	0.406	0.166	0.535	0.345

Table 3
Exact test of population differentiation based on allele frequencies

Population pair	D10S1248	D14S1434	D22S1045	D1S1677	D2S441	D4S2364
Chinese–Malay	0.073	0.393	0.339	0.541	*0.000*	0.162
Chinese–Indian	*0.000*	*0.022*	*0.000*	0.067	*0.000*	0.156
Malay–Indian	*0.012*	0.073	*0.000*	0.051	*0.000*	*0.004*

Values in italic represent significant difference with $p < 0.05$.

Results: Allele frequency shown in Table 1. Forensic parameters summarised in Table 2. Population differentiation test per locus was summarised in Table 3. Comparison of observed heterozygosity with other 15 common forensic STR in similar populations was shown in Table 4.

Quality control: Commercial DNA standard 9947 (Coriell Cell Repositories, NJ, USA), was genotyped as standard reference. Allelic ladder of mini01 was kindly provided by Coble and Butler [2]. A concordant study was carried out to ensure result reproducibility and accuracy. Approximately 6.2% and 4.8% of samples (34 and 26 samples) were re-genotyped for mini01 and mini02, respectively. All genotype results were in full concordance.

Analysis of data: Forensic statistical parameters were performed using the software PowerStatsV12 spreadsheet (http://www.promega.com/geneticidtools/powerststs/). Possible divergence from Hardy–Weinberg equilibrium was tested by exact test [3] based on 20,000 simulations (http://www.dbioinfo.org/). Population differentiation test using exact test was carried out with the programme Arlequin Version 2.000 [4].

Access to data: Through e-mail from corresponding author.

Other remarks: The observed allele sizes ranged between 71 and 121 bp in our system. No markers demonstrated significant deviation from Hardy–Weinberg equilibrium using the exact test.

This allele distribution of all six miniSTR loci proved that they are equally polymorphic in all three Singapore populations, and their heterozygosity values are comparable to other world populations [2,5]. The observed heterozygosity ranges from 0.600 to 0.816, 0.610 to 0.758 and 0.640 to 0.764 for Chinese, Malay and Indian, respectively. D4S2364 being the least polymorphic marker, but still achieved a heterozygosity of >0.6. The combined random match probability (RMP) of the six miniSTR were calculated to be 4.6×10^{-6}, 3.5×10^{-6} and 4.2×10^{-6}, while the combined power of exclusion were 97.77%, 96.68% and 97.55% for Chinese, Malay and Indian, respectively.

The results of population differentiation test for each population pair per locus was summarised in Table 3. The locus D2S441 showed the most significant differentiation for all three population pairs, while D1S1677 is not significant for all. The Chinese–Malay pair has no significant differentiation for all markers except D2S441.

Two novel microvariant alleles were observed in this study. These included allele 13.1 of D1S1677 in a Malay sample, and allele 17.3 of D14S1434 in a Chinese sample. These alleles were reproducible, and were confirmed by re-genotyping using a second set of flanking primer pairs that included the miniPCR amplicon [2].

A comparison of the observed heterozygosity values with 15 other STR obtained from earlier study genotyping similar populations [6] was summarised in Table 3. Three of the miniSTR has comparatively medium level of heterozygosity, while the other three miniSTR have lower heterozygosity values. The main benefit of miniSTR is its small PCR amplicon size that increases the likehood of amplifying degraded DNA. Future work would be to develop miniSTR primer pairs for those STR loci that showed high heterozygosity in local populations.

This paper follows the guidelines for publication of population data requested by the journal [7].

Table 4
Comparison of observed heterozygosity values of six miniSTR loci (in bold) and 15 STR loci from similar ethnic populations in Singapore

Marker	Observed heterozygosity			
	Chinese	Malay	Indian	Average
Penta E	0.897	0.876	0.938	0.904
FGA	0.875	0.857	0.847	0.860
D18S51	0.864	0.814	0.825	0.834
D8S1179	0.859	0.826	0.814	0.833
D21S11	0.853	0.826	0.802	0.827
vWA	0.826	0.752	0.831	0.803
Penta D	0.837	0.783	0.780	0.800
D5S818	0.832	0.776	0.785	0.798
D16S539	0.783	0.770	0.825	0.793
D13S317	0.750	0.764	0.836	0.783
D7S820	0.766	0.783	0.791	0.780
D22S1045-mini01	**0.816**	**0.742**	**0.719**	**0.759**
D10S1248-mini01	**0.795**	**0.720**	**0.736**	**0.750**
TH01	0.685	0.720	0.790	0.732
D2S441-mini02	**0.719**	**0.758**	**0.697**	**0.725**
CSF1P0	0.766	0.671	0.706	0.714
D3S1358	0.717	0.643	0.780	0.713
D14S1434-mini01	**0.676**	**0.714**	**0.747**	**0.712**
D1S1677-min02	**0.643**	**0.632**	**0.764**	**0.680**
TPOX	0.560	0.646	0.706	0.637
D4S2364-mini02	**0.600**	**0.610**	**0.640**	**0.617**

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