## The development of a 20-locus fluorescent multiplex system as valuable tools for national DNA database **Supplementary data**

Table S1-S9

 Table S1

 The uniform upstream primer and a series of downstream primers sequences used for internal size standard.

Primer	Sequence (5'-3')
F	GGCGAAACCCGACAGGACTA
75R	TCGGAACAGGAGAGCGCA
80R	CAGGGTCGGAACAGGAGAG
100R	GACAGGTATCCGGTAAGCGG
140R	GCTATGAGAAAGCGCCACG
160R	CTGAGATACCTACAGCGTGAGC
175R	ACGACCTACACCGAACTGAGA
180R	AGCGAACGACCTACACCGA
200R	GTGCACACAGCCCAGCTTG
225R	AGCGGTCGGGCTGAACGGGGGTT
250R	AGACGATAGTTACCGGAT
275R	GTCGTGTCTTACCGGGT
300R	CGAGAACTGCTGCCAGTGGCG
330R	CTACATACCTCGCTCTGCTAATCC
360R	GCCACCACTTCAAGAACTCTGT
390R	ATACTGTTCTTCTAGTGTAGCCGTAG
445R	GAGCTACCAACTCTTTTCC
500R	CTGCTTGCAAACAAAAAACC

F: Forward primer; R: Reverse primer; the number before R: the amplicon size.

## Supplementary data Table S2

The PCR components and thermal cycling parameters of internal size standard

PCR components	Volume (μl)
ddH <sub>2</sub> O	6
10×PCR mix	1
dNTP	0.8
Upstream primer	0.25
Downstream primers	1
pUC18	1
Tag polymerase	0.2
Total	10

Thermal cycling parameters:

- denaturation for 2 min at 94°C
  amplification for 30 cycles of 20 s at 94°C, 40 s at 58°C, 40 s at 72°C
  extension for 10 min at 70°C

## Supplementary data Table S3

The plasmid genomic DNA sequence

-	The plasmid genomic DNA sequence										
Positio	ons of various elements										
Vector	r size (bp)			2686							
	ole cloning site		399-455								
	α-peptide			146-469							
	cillin resistance gene			1626-2486							
pUC o	rigin			867-1455							
1	TCGCGCGTTT	CGGTGATGAC	GGTGAAAACC	TCTGACACAT	GCAGCTCCCG						
51	GAGACGGTCA	CAGCTTGTCT	GTAAGCGGAT	GCCGGGAGCA	GACAAGCCCG						
101	TCAGGGCGCG	TCAGCGGGTG	TTGGCGGGTG	TCGGGGCTGG	CTTAACTATG						
151	CGGCATCAGA	GCAGATTGTA	CTGAGAGTGC	ACCATATGCG	GTGTGAAATA						
201	CCGCACAGAT	GCGTAAGGAG	AAAATACCGC	ATCAGGCGCC	ATTCGCCATT						
251	CAGGCTGCGC	AACTGTTGGG	AAGGGCGATC	GGTGCGGGCC	TCTTCGCTAT						
301	TACGCCAGCT	GGCGAAAGGG	GGATGTGCTG	CAAGGCGATT	AAGTTGGGTA						
351	ACGCCAGGGT	TTTCCCAGTC	ACGACGTTGT	AAAACGACGG	CCAGTGCCAA						
401	GCTTGCATGC	CTGCAGGTCG	ACTCTAGAGG	ATCCCCGGGT	ACCGAGCTCG						
451	AATTCGTAAT	CATGGTCATA	GCTGTTTCCT	GTGTGAAATT	GTTATCCGCT						
501	CACAATTCCA	CACAACATAC	GAGCCGGAAG	CATAAAGTGT	AAAGCCTGGG						
551	GTGCCTAATG	AGTGAGCTAA	CTCACATTAA	TTGCGTTGCG	CTCACTGCCC						
601	GCTTTCCAGT	CGGGAAACCT	GTCGTGCCAG	CTGCATTAAT	GAATCGGCCA						
651	ACGCGCGGGG	AGAGGCGGTT	TGCGTATTGG	GCGCTCTTCC	GCTTCCTCGC						
701	TCACTGACTC	GCTGCGCTCG	GTCGTTCGGC	TGCGGCGAGC	GGTATCAGCT						
751	CACTCAAAGG	CGGTAATACG	GTTATCCACA	GAATCAGGGG	ATAACGCAGG						
801	AAAGAACATG	TGAGCAAAAG	GCCAGCAAAA	GGCCAGGAAC	CGTAAAAAGG						
851	CCGCGTTGCT	GGCGTTTTTC	CATAGGCTCC	GCCCCCTGA	CGAGCATCAC						
	AAAAATCGAC	GCTCAAGTCA	GAGGT <mark>GGCGA</mark>	AACCCGACAG							
901 951	ATACCAGGCG	TTTCCCCCTG	GAAGCTCCCT		GACTATAAAG						
				CGTGCGCTCT	CCTGTTCCGA						
1001	CCCTGCCGCT	TACCGGATAC	CTGTCCGCCT	TTCTCCCTTC	GGGAAGCGTG						
1051	GCGCTTTCTC	ATAGCTCACG	CTGTAGGTAT	CTCAGTTCGG	TGTAGGTCGT						
1101	TCGCTCCAAG	CTGGGCTGTG	TGCACGAACC	CCCCGTTCAG	CCCGACCGCT						
1151	GCGCCTTATC	CGGTAACTAT	CGTCTTGAGT	CCAACCGGT	AAGACACGAC						
1201	TTATCGCCAC	TGGCAGCAGC	CACTGGTAAC	AGGATTAGCA	GAGCGAGGTA						
1251	TGTAGGCGGT	GCTACAGAGT	TCTTGAAGTG	GTGGCCTAAC	TACGGCTACA						
1301	CTAGAAGAAC	AGTATTTGGT	ATCTGCGCTC	TGCTGAAGCC	AGTTACCTTC						
1351	GGAAAAAGAG	TTGGTAGCTC	TTGATCCGGC	AAACAAACCA	CCGCTGGTAG						
1401	CGGTGGTTTT	TTTGTTTGCA	AGCAGCAGAT	TACGCGCAGA	AAAAAAGGAT						
1451	CTCAAGAAGA	TCCTTTGATC	TTTTCTACGG	GGTCTGACGC	TCAGTGGAAC						
1501	GAAAACTCAC	GTTAAGGGAT	TTTGGTCATG	AGATTATCAA	AAAGGATCTT						
1551	CACCTAGATC	CTTTTAAATT	AAAAATGAAG	TTTTAAATCA	ATCTAAAGTA						
1601	TATATGAGTA	AACTTGGTCT	GACAGTTACC	AATGCTTAAT	CAGTGAGGCA						
1651	CCTATCTCAG	CGATCTGTCT	ATTTCGTTCA	TCCATAGTTG	CCTGACTCCC						
1701	CGTCGTGTAG	ATAACTACGA	TACGGGAGGG	CTTACCATCT	GGCCCCAGTG						
1751	CTGCAATGAT	ACCGCGAGAC	CCACGCTCAC	CGGCTCCAGA	TTTATCAGCA						
1801	ATAAACCAGC	CAGCCGGAAG	GGCCGAGCGC	AGAAGTGGTC	CTGCAACTTT						
1851	ATCCGCCTCC	ATCCAGTCTA	TTAATTGTTG	CCGGGAAGCT	AGAGTAAGTA						
1901	GTTCGCCAGT	TAATAGTTTG	CGCAACGTTG	TTGCCATTGC	TACAGGCATC						
1951	GTGGTGTCAC	GCTCGTCGTT	TGGTATGGCT	TCATTCAGCT	CCGGTTCCCA						
2001	ACGATCAAGG	CGAGTTACAT	GATCCCCCAT	GTTGTGCAAA	AAAGCGGTTA						
2051	GCTCCTTCGG	TCCTCCGATC	GTTGTCAGAA	GTAAGTTGGC	CGCAGTGTTA						
2101	TCACTCATGG	TTATGGCAGC	ACTGCATAAT	TCTCTTACTG	TCATGCCATC						
2151	CGTAAGATGC	TTTTCTGTGA	CTGGTGAGTA	CTCAACCAAG	TCATTCTGAG						
2201	AATAGTGTAT	GCGGCGACCG	AGTTGCTCTT	GCCCGGCGTC	AATACGGGAT						
2251	AATACCGCGC	CACATAGCAG	AACTTTAAAA	GTGCTCATCA	TTGGAAAACG						
2301	TTCTTCGGGG	CGAAAACTCT	CAAGGATCTT	ACCGCTGTTG	AGATCCAGTT						
2351	CGATGTAACC	CACTCGTGCA	CCCAACTGAT	CTTCAGCATC	TTTTACTTTC						
2401	ACCAGCGTTT	CTGGGTGAGC	AAAAACAGGA	AGGCAAAATG	CCGCAAAAA						
2451	GGGAATAAGG	GCGACACGGA	AATGTTGAAT	ACTCATACTC	TTCCTTTTTC						
2501	AATATTATTG	AAGCATTTAT	CAGGGTTATT	GTCTCATGAG	CGGATACATA						
2551	TTTGAATGTA	TTTAGAAAAA	TAAACAAATA	GGGGTTCCGC	GCACATTTCC						
2601	CCGAAAAGTG	CCACCTGACG	TCTAAGAAAA	CATTATTATC	ATGACATTAA						
2651	CCTATAAAAA	TAGGCGTATC	ACGAGGCCCT	TTCGTC	onemina						
2001	CCIMIAAAAA	MOGCOTATE	110011000001								

## Supplementary data Table S4

Allele frequencies and genetic parameters for 19 STR loci in Northern Chinese Han population (n=3000).

	Allele frequencies and genetic parameters for 19 STR loci in Northern Chinese Han population (n=3000).																		
Allele	TPOX	D16S539	TH01	CFS1PO	D13S317	D3S1358	D21S11	D18S51	D6S1043	Penta E	FGA	D2S1338	D5S818	D8S1179	Penta D	D7S820	vWA	D12S391	D19S433
4			0.0002							0.0465					0.0002				
5 6	0.0002		0.0003 0.0979							0.0465 0.0002			0.0005		0.0002 0.0040				
7	0.0002		0.0979	0.0005	0.0015			0.0003		0.0002			0.0003		0.0040	0.0017			
8	0.5072	0.0085	0.2044	0.0003	0.2699			0.0003	0.0002	0.0027			0.0133	0.0008	0.0055	0.0017			
9	0.1238	0.2813	0.5220	0.0524	0.2077			0.0002	0.0002	0.0082			0.0687	0.0003	0.3121	0.1500			
10	0.0265	0.1231	0.0172	0.2361	0.1440			0.0002	0.0327	0.0417			0.1970	0.0944	0.1178	0.1668	0.0002		0.0007
11	0.3103	0.2456	0.0003	0.2404	0.2289			0.0037	0.0976	0.1225			0.3407	0.0744	0.1660	0.3365	0.0002		0.0027
12	0.0302	0.2135		0.3844	0.1640	0.0007		0.0324	0.1336	0.1103			0.2171	0.1378	0.1834	0.2514			0.0409
13	0.0012	0.1121		0.0722	0.0375	0.0003		0.2067	0.1396	0.0501	0.0002	0.0002	0.1461	0.2284	0.1256	0.0392	0.0025		0.2956
14		0.0145		0.0103	0.0097	0.0432		0.2112	0.1366	0.0891			0.0088	0.1937	0.0307	0.0027	0.2549	0.0007	0.2484
15		0.0013		0.0015	0.0005	0.3629		0.1775	0.0158	0.1044	0.0002		0.0017	0.1760	0.0083	0.0002	0.0309	0.0145	0.0659
16						0.3247		0.1260	0.0037	0.0871	0.0002	0.0032	0.0002	0.0756	0.0007		0.1747	0.0055	0.0127
17						0.1902		0.0742	0.0397	0.0839	0.0018	0.0427		0.0148			0.2477	0.0894	0.0007
18						0.0722		0.0387	0.1752	0.0756	0.0227	0.0812		0.0030			0.1867	0.2319	0.0003
19						0.0052		0.0415	0.1545	0.0612	0.0475	0.1104		0.0003			0.0804	0.2241	
20						0.0005		0.0320	0.0559	0.0484	0.0487	0.0782					0.0202 0.0013	0.1732	
21 22								0.0204 0.0200	0.0073 0.0005	0.0277 0.0157	0.0971 0.1647	0.0167 0.0350					0.0013	0.1069 0.0836	
23								0.0200	0.0003	0.0137	0.1047	0.0330					0.0002	0.0830	
24								0.0037	0.0007	0.0100	0.2370	0.1300					0.0002	0.0429	
25								0.0038		0.0018	0.1049	0.0444						0.0070	
26							0.0002	0.0003		0.0015	0.0494	0.0062						0.0013	
27							0.0032			0.0003	0.0070	0.0028						0.0005	
28							0.0472				0.0013	0.0153							
29							0.2553				0.0005	0.0858							
30							0.3010				0.0002	0.0886							
31							0.0976					0.0302							
32							0.0279					0.0098							
33							0.0042					0.0008							
34							0.0007					0.0002				0.0010			
9.1 9.2																0.0018			0.0003
9.2			0.0414																0.0003
10.1			0.0414													0.0002			
10.3																0.0002			
11.2															0.0002	*****			0.0005
12.1																0.0002			
12.2																			0.0038
13.2																			0.0397
14.1										0.0002									
14.2						0.000											0.000=		0.1089
15.2						0.0002		0.0002			0.0002						0.0002		0.1408
16.2								0.0002			0.0002								0.0337

17.2								0.0002											0.0035
17.3									0.0002										
18.2									0.0005		0.0002								0.0008
18.3										0.0002									
19.1										0.0002									
19.2																	0.0002	0.0007	
19.3											0.0002							0.0007	
20.2									0.0002		0.0003								
20.3									0.0002		0.0020								
21.2 21.3									0.0035		0.0020								
22.2									0.0033		0.0065								
22.2									0.0003		0.0003								
23.2									0.0003		0.0098								
23.3									0.0002		0.0076								
24.2									0.0002		0.0065								
25.2											0.0003								
26.2											0.0005								
27.2											0.0002								
28.2							0.0072				0.0002	0.0023							
29.2							0.0017					0.0002							
30.2							0.0125					0.0048							
30.3							0.0055					0.0018							
31.2							0.0774					0.0260							
32.2							0.1180					0.0359							
33.1							0.0002												
33.2							0.0355					0.0112							
34.1							0.0002												
34.2							0.0042					0.0015							
35.2							0.0007												
••	0.6276	0.5001	0.6402		0.5005	0.5115	0.0005	0.0540	0.0710	0.0102	0.0610	0.0550		0.0205	0.0010	0.7010	0.0060	0.0500	0.0020
He	0.6276	0.7931	0.6403	0.7277	0.7935	0.7117	0.8205	0.8542	0.8719	0.9193	0.8612	0.8559	0.7798	0.8385	0.8018	0.7818	0.8068	0.8509	0.8038
DP	0.8012	0.9214	0.8219	0.8829	0.9330	0.8732	0.9407	0.9618	0.9705	0.9875	0.9619	0.9774	0.9125	0.9541	0.9384	0.9132	0.9298	0.9530	0.9412
PIC	0.5680	0.7539	0.5945	0.6864	0.7764	0.6708	0.7871	0.8372	0.8605	0.9142	0.8369	0.9089	0.7371	0.8199	0.7838	0.7374	0.7708	0.8172	0.7863
PE	0.3254	0.5863	0.3421	0.4724	0.5870	0.4466	0.6376	0.7031	0.7384	0.8349	0.7170	0.7064	0.5621	0.6723	0.6024	0.5657	0.6117	0.6965	0.6061

He: Heterozygosity; DP: Discrimination power; PIC: Polymorphism information content; PE: Probability of exclusion

 $\begin{tabular}{ll} \textbf{Supplementary data} \\ \textbf{Table S5} \\ \end{tabular}$  The genotypes of sample A and sample B were used in mixture studies

Locus	Sample A	Sample B
D19S433	13/14	14/14.2
D5S818	12/12	10/13
<u>D21S11</u>	29/31.2	28/31
D18S51	16/18	17/17
D6S1043	12/20	21/21
D3S1358	17/18	15/16
D13S317	9/11	13/13
<u>D7S820</u>	8/11	10/10
D16S539	9/13	8/11
CSF1PO	12/12	10/12
Penta D	12/13	3.2/9
Amel	X/Y	X/Y
vWA	16/19	16/19
D8S1179	14/15	14/15
<u>TPOX</u>	11/11	8/13
Penta E	7/14	5/13
TH01	6/9.3	6/7
D12S391	18/23	18/19
D2S1138	22/25	22/22
<u>FGA</u>	20/23	19/27

<u>Underline</u> of locus indicates that alleles between sample A and sample B are totally different.