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A population study of three Y-STR loci by multiplexing in Han population in Chengdu, China

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Abstract. *Background*: it is necessary to enhance the efficiency of amplification of Y-STR in according with the progress in forensic science. Methods: Using a multiplex of Y-STRs and amplify simultaneously three Y-STRs loci. *Results*: A multiplexing system of three Y-STR loci (DYS390, DYS391 and DYS393) is successfully established followed by a population genetic study of Han population in Chengdu, China. *Conclusions*: The diversity of haplotype is 0.8965, the value of discrimination and the chance of exclusion chance is 0.8965 with the standard error 0.0081. This established system is one of the good tools in personal identification and genetic study.

Keywords: Forensic science; Y-STRs; DYS390; DYS391; DYS393; Multiplexing; Population study

1. Introduction

Y-STRs systems have been used in paternity testing of male offspring, personal identification and population genetic study, The non-pseudoautosomal part of the Y chromosome is inherited only along male lineages. A single Y-STR locus can only provide a limited information. There is no doubt that multiplexing of one more STR loci will provide more informations. We have constructed a multiplex system and amplified three Y-STRs loci simultaneously, followed by non-denaturing polyacrylamide gel electrophoresis and silver staining.

2. Survey methodology

EDTA-blood samples were collected from 105 unrelated male of Han population in Chengdu of China. DNA was extracted using Chelex-100 method [1].

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Table 1
The primer sequences of DYS390, DYS391 and DYS393

Loci	Allele	Primer sequences
DYS390	21–26	P1: 5′-TAT ATT TTA CAC ATT TTT GGC CC-3′
		P2: 5′-GAC AGT AAA ATG AAC ACA TTG C-3′
DYS391	9–11	P1: 5′-CTA TTC ATT CAA TCA TAC ACC CA-3′
		P2: 5′-CTG GGA ATA AAA TCT CCC TGG TTG GGA G-3′
DYS393	12–16	P1: 5′-GTG GTC TTC TAC TTG TGT CAA TAC AG-3′
		P2: 5′-AAC TCA AGT CCA AAA AAT GAG G-3′

In order to reduce the unspecific amplification products, the primers of DYS391 and DYS393 were modified respectively by Leonor et al. [2] and Berit et al. [3].The primers of DYS390 were the same as these in the GDB [4] (Table 1).

The PCR amplifications was conducted in a thermal cycler (HEIMA 480) in a 20-μl reaction volume, which included 50–100 ng of DNA template, 2 μl 10 × PCR reaction buffer, 1.25 μl $MgCl_2$ (25 mmol/l), 1.5 μl DYS390 primers (12.5 pmol/μl), 1.0 μl DYS391 primers (12.5 pmol/μl), 1.2 μl DYS393 primers (12.5 pmol/μl), 1.25 U Taq DNA polymerase.

The PCR conditions were 94 °C for 3 min, followed by 35 cycles at 94 °C for 35 sec, 57 °C for 35 s and 72 °C for 55 s, then extension at 72 °C for another 5 min.

A total of 3 μl PCR products was analyzed by non-denaturing polyacrylamide gel electrophoresis ($T = 6\%$, $C = 5\%$) with discontinuous buffer system and visualized by silver staining [5].

Table 2
Haplotype distribution of DYS391/DYS390/DYS393 of Han population in Chengdu, China (n = 105)

Haplotype	Frequency	Number	Haplotype	Frequency	Number
9/23/12	0.009524	1	10/25/13	0.028571	3
9/25/12	0.009524	1	10/25/14	0.009524	1
9/25/13	0.019048	2	11/23/12	0.028571	3
10/21/15	0.009524	1	11/23/13	0.066667	7
10/22/12	0.019048	2	11/23/14	0.009524	1
10/22/13	0.009524	1	11/23/15	0.019048	2
10/22/14	0.009524	1	11/23/16	0.009524	1
10/23/12	0.171429	18	11/24/12	0.019048	2
10/23/13	0.07619	8	11/24/13	0.047619	5
10/23/14	0.038095	4	11/24/14	0.019048	2
10/23/15	0.028571	3	11/25/12	0.028571	3
10/24/12	0.142857	15	11/25/13	0.019048	2
10/24/13	0.066667	7	11/25/14	0.009524	1
10/24/14	0.028571	3	11/26/14	0.009524	1
10/25/12	0.038095	4			
Discrimination power = 0.8965			Exclusion chance = 0.8965		
Diversity = 0.8965 ± 0.0081					

3. Results

The haplotype diversity and standard error were calculated in accordance with Hou's method [6]. The formula for calculating the gene diversity: $h = n\ (1 - \Sigma x^2)/(n - 1)$, where n is the number of individuals tested, h is the gene diversity, x is the frequencies of alleles.

The formula calculating standard error (S.E.): $\text{S.E.} = \{2\{\Sigma x^3 - (\Sigma x^2)^2\}/n\}^{1/2}$.

The Y chromosome haplotypes of 105 unrelated males from Han population in Chengdu, China are presented in Table 2.

4. Discussion

In case of multiplexing STRs, the optimal ratio of primer pairs used in the multiple system is very important. In this study, the best proportion was 1.5/1.0/1.2 (DYS390/DYS391/DYS393). The $MgCl_2$ is an another important factor to affect the results of the PCR reactions. About 1.25 μl $MgCl_2$ (25 mmol/l) in a 20-μl reaction volume was the best. This method is reliable, easy to perform, time saving, less consuming either samples or reagents.

In our population study, the diversity of haplotype was 0.8965, the value of discrimination and the chance of exclusion chance was 0.8965 with the standard error 0.0081. Our results demonstrated that these three Y-STRs can provide more informations in Han population in Chengdu, China. The application of this multiplex system is helpful in resolving some forensic science cases, such as paternity testing only with son, personal identification, especially examination of mixture stain in rape cases and in anthropological investigation cases.

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