

POPULATION GENETICS OF THREE STR POLYMORPHISMS IN A CHINESE POPULATION

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INTRODUCTION

Short tandem repeats (STRs) belong to a type of DNA polymorphisms with core sequences of a few base pairs in length. They are analysed using the polymerase chain reaction (PCR) and show variable allele lengths from 100 to 500 bp. The PCR products have to be separated by polyacrylamide gel electrophoresis with a resolution of one base pair (1). STRs offer three obvious advantages for forensic purposes. Firstly, the allele classifications can be based upon the number of tandem repeats relative to an allele ladder, so that correct typing is permitted. Secondly, samples containing very small amounts of DNA can be analysed. Thirdly, the amplification of alleles is also possible with highly degraded DNA. Therefore, we analysed 3 STR systems (VWA, HUMTH01 and FES)(2-4) to obtain preliminary allele frequency data for a Chinese population.

MATERIALS AND METHODS

EDTA-blood samples were collected from 121 unrelated Chinese individuals in Chengdu, Sichuan province, PR China. DNA was extracted from EDTA-blood by the Chelex method(5). The amplification was carried out with the following primers:

VWA (Kimpton et al.1992)(2)	5'-CCC TAG TGG ATG ATA AGA ATA ATC
	5'-GGA CAG ATG ATA AAT ACA TAG GAT GGA TGG
HUMTH01 (CRSE)	5'-GTG GGC TGA AAA GCT CCC GAT TAT
	5'-GTG ATT CCC ATT GGC CTG TTC CTC
FES (CRSE)	5'-GGG ATT TCC CTA TGG ATT GG
	5'-GCG AAA GAA TGA GAC TAC AT

Each amplification sample contained 2-40 ng human genomic DNA, 1xTaq buffer (Promega), 1.5mM MgCl₂, 150µM of each nucleotide, 1U Taq polymerase (Promega) and 0.25 µM of each primer. A total of 28 cycles was carried out in a Thermocycler (Biozym) with denaturation for 1 min at 94°C, annealing for 1 min at 60°C (for FES at 54°C), and extension for 2 min at 72°C.

Electrophoretical separation of the PCR products was performed in polyacrylamide gels (750µm thick, horizontal) with following conditions:

VWA	7.2% T, 3% C, 24 mM leading buffer.
HUMTH01	7.2% T, 3% C, 31 mM leading buffer.
FES	7.0% T, 3% C, 24 mM leading buffer.

A discontinuous buffer system was employed, Tris-Sulfate, pH 9.0 as leading buffer and 280mM Tris-Borate, pH 9.0 as tracking buffer. The separation distance was 20 cm and electrophoresis was stopped when the bromophenol blue front had reached the anodal end of the gel. The gels were silverstained according to Allen et al.(6).

Allele determination was carried out by comparison with the allele ladders of VWA, HUMTH01 and FES that have been constructed and evaluated by the Central Research and Support Establishment (CRSE) of the Forensic Science Service, UK.

The test for Hardy-Weinberg equilibrium was carried out using chi square test. The expected heterozygosity was calculated according to the equation $h=(1-\sum X_i^2)(n/n-1)$, where h =expected heterozygosity, X_i =allele frequencies and n =the number of alleles(7).

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