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Newly designed multiplex amplification and genotyping system at four pentanucleotide repeat STR loci useful for degraded mixed DNA specimens

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Abstract. We constructed a multiplex PCR and genotyping system selecting four pentanucleotide STR loci (Penta E, Penta D, Penta B and D10S2325) with multi-colored fluorescently labeled primer sets that are newly designed in order to genotype from mixed specimens, especially for degraded DNA samples, of which the amplicon sizes are smaller than about 200 bp. The allele frequencies at these four STR loci were calculated in a Japanese population. Sequence analysis was also performed for all alleles at these all STR loci observed in the Japanese population, and the allele distributions at those four loci did not deviated from HWE. The heterozygosities and the power of discriminations (PD) at all the four loci were more than 0.80 and 0.93, respectively. Furthermore, the present study provides a statistical standard to decide whether a one-repeat small peak from a main peak is the stutter peak or the peak originated from mixed individual sample.

Keywords: STR; Pentanucleotide; Multiplex; Mixed stains

1. Introduction

Short tandem repeat (STR) markers with tetranucleotide repeat are globally utilized for personal identification and kinship analysis in forensic field, and give us very useful information in almost all practical cases. In case of mixed samples, however, the ‘stutter peaks’ sometimes make it difficult to interpret whether they are exact ‘stutter peaks’ or minor peaks originating from another individual, because the ratio of the stutter peak height to a main peak height is more than about 10% of stutter percentage for

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tetranucleotide STRs. In general, it is said that the stutter percentages become lower with the more numbers of repeat units [1–3].

In the present study, we selected four pentanucleotide STR loci, newly designed primer sets for degraded DNA samples, and constructed a multiplex PCR and genotyping system with fluorescently labeled primers. We also calculated the allele frequencies at these four loci in a Japanese population, and estimated the discriminating power. Furthermore, we analyzed the stutter percentage at each locus, and discussed about a potential application for forensic fields.

2. Materials and methods

In pentanucleotide STR loci, we selected a total of four loci, Penta E, Penta D, D10S2325, of which heterozygosities were previously reported as more than 0.80, and Penta B, which is included in the Penta BEC Multiplex kit released by Promega. Each primer set for those four loci were designed and labeled with multicolor fluorescent dyes, and constructed a multiplex PCR and genotyping system of which the amplicon sizes are smaller than about 200 bp. Sequence analysis was performed for all alleles of all STR loci observed in a Japanese

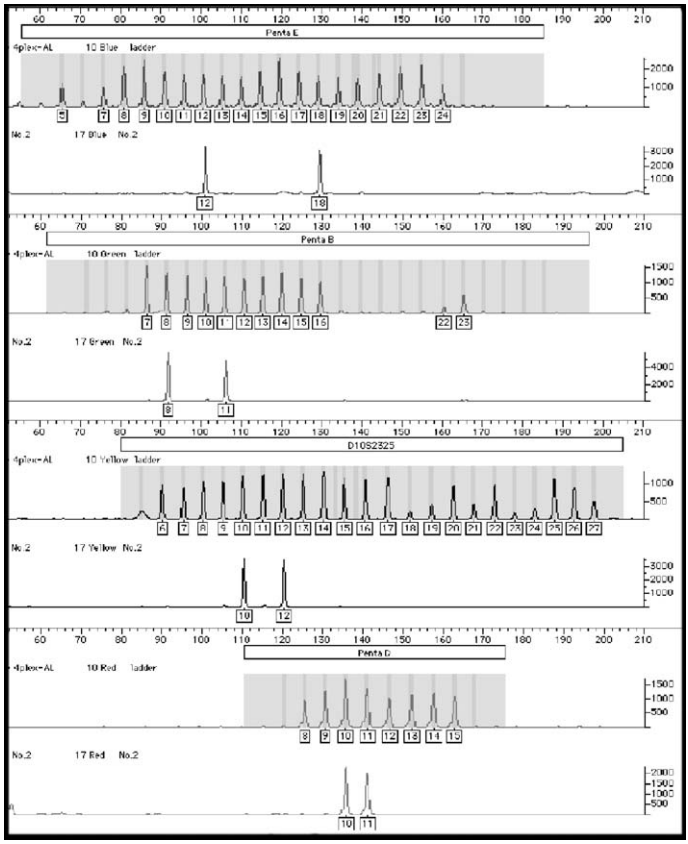

Fig. 1. An example of Genotyper plot using the present multiplex system.

population, and also the allelic ladder markers with these alleles inserted into the plasmids in a commercially available kit were constructed for semi-automated genotyping by making a template of Genotyper 2.5 software.

Using the present multiplex system, 300 unrelated Japanese (Nagoya City) were genotyped with written informed consent.

Tests for Hardy-Weinberg equilibrium (HWE) were carried out using a homozygosity test, a likelihood ratio test and an exact test. Some statistical properties for discriminating power were calculated.

Furthermore, the stutter percentages at all alleles for these four loci, where stutter peak heights were more than 50 RFU, were calculated.

3. Results and discussion

We constructed a multiplex PCR amplification and genotyping system for four pentanucleotide STR loci, Penta E, Penta D, D10S2325, and Penta B, of which amplicon sizes were less than about 200 bp (Fig. 1). Sequence analysis revealed that no nucleotide substitution in repeat regions at all four loci, but that one substitution T to A upstream from repetitive region and two substitutions A to G and G to A down stream in the flanking regions at D10S2325 loci.

The allele frequencies at those four loci were calculated from 300 Japanese. Three tests for Hardy-Weiberg equilibrium (HWE) were performed, and the allele distributions at those four loci did not deviate from HWE. The heterozygosities and the power of discriminations (PD) at all four loci were more than 0.80 and 0.93, respectively. The combined PD and MEC (mean exclusion chance) were 0.9999962 and 0.9900966, respectively.

The stutter percentages at all alleles for these four loci, where stutter peak heights were more than 50 RFU, were calculated, and it was found that the stutter percentages were almost directly proportional to the numbers of repeats at each locus, and that almost all the stutter percentages were distributed within the regression lines from ± 3 times the values of SD (standard deviation) of each allele for each locus. Accordingly, this could be a statistical standard to decide whether a one-repeat small peak from a main peak is the stutter peak or the peak originated from mixed individual sample. It was suggested that this system is one of the useful multiplex typing system, especially for mixed DNA specimens.

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