Construction of New Quadriplex AMP-FLP Systems for Autosomal and Sex Chromosomal STR Loci

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We introduce the development of three quadriplex systems, two of them are autosomal (D5S818-D13S737-D19S253-D3S2400, D2S1371-D8S1477-D12S391-D20S470) and the other is sex-linked (DYS390-DYS389I-HPRTB-DYS389 II). For efficient forensic case work and the construction of a national DNA database in Korea, it is necessary to use multiplex STR systems which fit the Korean population.

As a preliminary study, we have evaluated over 30 STR loci for suitable markers some of which are universally used and some are not yet. Allelic distribution in the Korean population was surveyed for each locus and validated through statistical analyses. First, we constructed three triplex STR systems with high discriminating power and stability, which are D3S1744-D12S391-D20S470, D5S818-D13S317-D19S253, and D8S1132-D13S325-D3S2106. These systems were designed for denaturing PAGE followed by silver staining and now are currently used as DNA typing systems in our laboratory. All the repeating units and the size of each locus was determined by nucleotide sequence analysis using an ABI® 377 DNA sequencer. In spite of their usefulness, triplex systems have some overlapping chromosomal loci, for example, D13S317 vs. D13S325 and D3S1744 vs. D3S2406. As it is desirable to avoid chromosomal overlapping in DNA database systems, we have decided to develop an advanced quadriplex system.

Eight loci which are not overlapped were selected. Through PCR optimization and ladder construction, two quadriplex STR systems described above were developed and sequence analyzed to verify the number of repeats of each locus. Validation study showed that these two systems were useful to not only fresh samples but also complicated forensic samples. The average discrimination power was much higher than that of commercially available multiplex typing kits in the Korean population. For example, D3S2406 proved to be extremely useful with a fairly even allelic distribution and higher PD value than VNTR, D1S80. Now, research on an automated typing system using fluorescent dye labeled primer is in process.

We also present a quadriplex system for gender determination. Amelogenin typing is a very useful tool for forensic gender typing with its high sensitivity. But in the case of mixed forensic samples, it is hard to interpret the result. Two Y linked markers (DYS389, DYS390) and one X linked marker (HPRTB) were selected and consequently a quadriplex system was made which enables the correct discrimination between male and female in one PCR. In case of no band on a gel with the Y-marker alone, it is impossible to interpret if absence of band was caused from DNA degradation or a female sample. Besides these advantages, the constructed system can be efficiently used for the study of paternal inheritance which is meaningful in Korea where lineage is regarded as important.