A REAL-TIME PCR ASSAY FOR THE ESTIMATION OF THE DNA DEGRADATION

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The degradation of DNA into smaller fragments is a special challenge to perform STR analysis from forensic samples. Real-time PCR assay is very useful tool for determining the amount of template DNA in STR analysis. As real-time PCR assay usually employs a single PCR amplicon size, using quantification results from degraded DNA to be added to PCR reaction mixture is not always appropriate for STR analysis. For the purpose of assessing the quality and quantity of degraded DNA, quantitative PCR assay using various sizes (50-207 bp) of amplicons in the same region of genomic DNA was developed. The nuclear target for quantification was human specific alpha satellites present in 500-1000 copies on chromosome 17 (D17Z1). In order to obtain various levels of degraded DNA, artificial degradation of human genomic DNA was achieved by deoxyribonuclease I (DNase I) digestion or photosensitization by visible light in the presence of methylene blue (MB)
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For highly degraded DNA samples, real-time PCR assays employing smaller amplicons would be more successfully performed than those employing larger amplicons. Therefore, it is expected that the ratio of the quantity of large amplicons to that of small amplicons (a degradation ratio) would provide a useful estimate for the degree of DNA degradation in each sample. The degradation ratio was calculated from the quantification of various sizes of amplicons in the same region of genomic DNA. STR analyses using Identifiler and/or MiniFiler kit were performed from each degraded DNA. In the same degradation method, the degradation ratios were related to the number of detected alleles in STR analysis. However, the correlation between the degradation ratio and the number of detected alleles were dependent on how it was degraded. The real-time PCR assay for quantification of multiple lengths of amplicons in the same region of genomic DNA could estimate the degree of DNA degradation based on the degradation ratio and, thus, would be helpful for selecting adequate STR analysis to obtain profiles successfully.