E47 An Evaluation of the Efficacy of Whole Genome Amplification (WGA) for Degraded DNA

Mitsuyo Machida, Tokyo Women's Medical University, Tokyo 162-8666, JAPAN; Kazuhiko Kibayashi, MD, Tokyo Women's Medical University, Tokyo 162-8666, JAPAN*

Learning Overview: After attending this presentation, attendees will understand the need for Whole Genome Amplification (WGA) prior to Short Tandem Repeat (STR) analysis using degraded DNA.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by demonstrating that WGA is a potential preamplification method for STR analysis of challenging DNA samples.

DNA in biological samples is often vulnerable to environmental factors, such as Ultraviolet (UV) irradiation, high temperature, high humidity levels, and microorganism degradation. STR profiling, the most commonly used method for forensic DNA identification, is difficult to analyze highly degraded DNA samples, since structural modification or fragmented DNA templates can affect downstream analyses. An increase in the number of starting templates may improve the success of STR profiling. One approach to increase the number of DNA templates is WGA. Although WGA has mainly been demonstrated in clinical applications, few studies have shown the ability to evaluate the availability of WGA for degraded DNA samples in forensics.

Therefore, a PCR-based WGA called modified Improved Primer Extension Preamplification (mIPEP) was performed prior to STR analysis using degraded DNA, since the PCR-based WGA method is less affected by DNA quantity and quality. The mIPEP method uses a 15-mer random-sequenced primer and low stringency annealing conditions to amplify larger templates of the genome. To assess the efficacy of the mIPEP method for degraded DNA, this study artificially degraded DNA using UV light for different durations and analyzed the STR profiling.

Saliva from four volunteers was previously dried onto filter papers. These samples were exposed to UV light (365nm) for 3, 7, and 14 days, and 1 month in duplicate. The DNA were extracted from UV-irradiated saliva samples. The mIPEP method was initiated using 5ng and 0.5ng of DNA. Following the mIPEP, STR analysis was performed using the AmpFℓSTR® Identifiler PCR Amplification Kit. This study was approved by the ethics committee at Tokyo Women's Medical University.

The average peak height of 16 STRs drastically decreased until seven days of irradiation, then gradually decreased until one month of irradiation without mIPEP. After performing mIPEP using 5ng of input DNA, the peak height was higher than that without and with mIPEP using 0.5ng of input DNA after seven days of irradiation. Successful STR profiling decreased with increasing duration, and 12% of the STR loci were shown after one month of irradiation without mIPEP. The number of detectable STRs with mIPEP using 0.5ng of DNA was similar to that without mIPEP after UVA irradiation. However, the number of detectable STRs with mIPEP using 5ng of DNA was greater than that without and with mIPEP using 0.5ng of input DNA after 14 days of irradiation. In addition, longer STR loci, such as D21S11, D7S820, CSF1PO, D2S1338, D18S51, and FGA, were difficult to recover despite performing mIPEP.

Hence, 5ng of input DNA is necessary for mIPEP to improve the success of STR profiling using highly degraded samples. Moreover, the difficult recovery of STR loci by mIPEP should be considered when analyzing STRs using degraded DNA.

Whole Genome Amplification, Short Tandem Repeat, Degraded DNA