B93 Quantitative Analysis of Botanical DNA by Real-Time PCR for Forensic Discrimination and Identification

Hitomi S. Kikkawa, 6-3-1 Kashiwanoha, Kashiwa, Chiba 277-0882, JAPAN; Kouichiro Tsuge, PhD, National Research Institute of Police Science, 6-3-1 Kashiwanoha, Kashiwa 277-0882, JAPAN; and Ritsuko Sugita, PhD, National Research Institute of Police Science, 6-3-1 Kashiwanoha, Kashiwa 277-0882, JAPAN*

After attending this presentation, attendees will better understand a new highly sensitive DNA quantification method for forensically relevant botanical samples.

This presentation will impact the forensic science community by describing a more accurate and effective examination method of DNA quantification for plant specimens. The goal of this study was to develop a Real-Time quantitative Polymerase Chain Reaction (RT-qPCR) analysis for a wide range of botanical species. The amount of genomic DNA required by this method is so small that it is possible to perform more exacting examinations afterward.

Botanical evidence found at a crime scene can provide valuable information in criminal investigations; however, the specimens can be fragmentary, which prevents accurate species identification based on morphological characteristics. Therefore, DNA-based identification has begun to be introduced into botanical forensic analysis.

DNA quantification is required for accurate and effective examination. If the result of the quantitative assay is unreliable, the potential for needing to use more DNA to repeat testing to avoid failure of the analysis is likely. The loss of DNA due to the need for reanalysis affects the ability to perform further important analyses. Furthermore, with short tandem repeat profiling, a common analysis method used in forensic identification, the amount of template DNA needs to be strictly controlled.

Conventional DNA quantification methods, such as Ultraviolet (UV) -visible spectrophotometry and fluorometry, often fail to quantify a dilute DNA solution correctly because of the influence of non-specific background noise. In addition, these methods require a relatively large amount of DNA. One of the significant problems with botanical evidence is the low yield of DNA from damaged samples. In such a case, conventional methods cannot be used to quantify the sample. In contrast, qPCR requires only a small amount of DNA. This method has the ability to estimate dilute DNA solutions accurately and is well-suited for enabling plant-specific quantification.

In this experiment, primers targeting the ribulose 1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene were used in a SYBR® green qPCR analysis method. The primers utilized were selected to identify a wide range of plant species previously identified by direct sequencing of specific loci.

To test the newly developed qPCR method, *Arabidopsis thaliana* genomic DNA was extracted from leaf, stem, and root material, then the qPCR was performed on serial dilutions of the extracted DNA. The lower detection limit was 1.6pg and a straight calibration curve was obtained in the range of 1.6pg to 1ng of DNA.

To examine whether quantification by qPCR can be generally used, this method was applied to other plant species. Five families, Asteraceae, Poaceae, Cyperaceae, Rosaceae, and Ranunculaceae, which are popular plants in Japan, were selected. It was determined that all samples could be analyzed successfully using the developed qPCR method.

In conclusion, it is demonstrated that qPCR analysis is a very effective method to quantify DNA from botanical samples. Moreover, this study raised the possibility that qPCR analysis can be applied to a wide range of plant species.

Forensic Botany, Quantitative Real-Time PCR, rbcL

Copyright 2015 by the AAFS. Unless stated otherwise, noncommercial *photocopying* of editorial published in this periodical is permitted by AAFS. Permission to reprint, publish, or otherwise reproduce such material in any form other than photocopying must be obtained by AAFS.