THE NIGHTS THE LIGHTS WENT OUT IN GERMANTOWN, AN AMPLIFICATION STUDY

R. Cline, C. Polhamus, L. Winebrenner, C. Leisy, A. Heller, M. Cicco, L. Grossweiler, J. E. Kokoszka, C. Word
Orchid Cellmark, Germantown, MD

Power outages that disrupt the amplification of samples normally result in re-extractions and/or re-amplifications. However forensic evidence is often of limited quantity, which can require consuming samples and thus prevent subsequent analysis. We have developed an approach for generating DNA profiles in such situations and present a validation study demonstrating that a complete DNA STR profile can be obtained after a thermal cycler has unexpectedly stopped during PCR amplification. We address whether a post-amplification agarose gel can be used to estimate total PCR cycles completed, and if samples exposed overnight at room temperature can be reliably typed. Lastly, this approach is applied to casework samples to illustrate its value in a forensic laboratory.

When a sudden loss of power to a thermal cycler occurs, the number of completed cycles is stored in the memory of more recently manufactured thermal cyclers such as the Perkin Elmer (PE) 9700. Unfortunately, this is not a feature of the older PE 480. Hence, for PE 480 users it is necessary to have a method to estimate the number of cycles completed. Three mock samples were amplified for 25, 26, 27, and 28 cycles, and the PCR products were analyzed on an agarose gel. Faint products were observed after 25 cycles of PCR, and products of increasing intensity were observed after 26, 27, and 28 cycles.

Following a power outage, samples can be exposed overnight at room temperature. To determine if such PCR products could be reliably typed, a mock power outage was performed on neat samples to replicate these conditions. The number of cycles completed prior to power interruption was known, and the appropriate number of additional cycles was completed to a total of 25, 26, 27, or 28. These samples were analyzed on an agarose gel, and the proper volume injected into an ABI 3100 Genetic Analyzer. A full profile was obtained when 26 to 28 total cycles of PCR were performed, and a nearly full profile with only 25 cycles.

To address the utility of this approach in the forensic laboratory, casework samples were tested. A power outage interrupted a recent amplification; fortunately, sufficient DNA was extracted and re-amplification was possible. The completed cycle number was estimated for the interrupted samples and additional cycles were performed. The interrupted samples were analyzed on the ABI 3100 PRISM® Genetic Analyzer and compared to the re-amplified samples. The results of this approach will be detailed in the presentation.