Precision and Accuracy in Fluorescent STR DNA Typing: Assessment of Benefits Imparted by the Use of Allelic Ladders With the Profiler Plus™ Kit

BenoTt Leclair and Ron M. Fourney
DNA Methods and Database, Central Forensic Laboratory, Royal Canadian Mounted Police, 1200 Vanier Parkway, Ottawa, Ontario, Canada, K1G 3M8

The field of Forensic DNA Typing has evolved considerably in recent years with the commercialization of highly discriminative megaplex kits that allow typing to be carried out in a single PCR amplification reaction. Some of these megaplexes feature several highly polymorphic STRs for which 1 and 2 base allele variants are routinely encountered in population studies. High resolution analytical platforms capable of precise measurements are thus required to discriminate such allele variants.

The Profiler Plus™ kit used in conjunction with the ABD 377 Prism® DNA Sequencer constitute the analytical platform selected by the RCMP for casework involving DNA typing. As precision and accuracy of DNA profiling are of paramount importance in a forensic setting, our in-house validation exercise has included the evaluation of the following:

1. the base calling precision of GeneScan® Analysis 2.1;
2. the accuracy of automated assignment by Genotyper® 2.0 of allele designations using allelic ladder data.

More specifically, we wanted to asses whether allelic ladders afforded any additional reliability in the assignment of alleles for a profile when compared to the use of allele-specific median values tabulated from population data.

A total of 468 individuals were genotyped with Profiler Plus™. We derived median base call and standard deviation values for all encountered alleles. We also measured the difference in size estimate for each peak vs. the size estimate of its gel-specific allelic ladder counter-part and calculated a modified standard deviation.

We have encountered very good precision on population data (medians with std. dev. of ±0.04 – 0.11) with the described platform, providing a 3SD window of ±0.33 base, well within the required ±0.5 base required to reliably discriminate 1 base variants. Automated assignment of allele designations was accurate, allowing rapid review of data and affording a common currency in data exchange. We have noted that allelic ladder lanes proved more sensitive to slight pipetting imprecisions in the preparation of the prescribed gel formulation. In some situations, allelic ladder peaks would appear shifted along with the matching Genotyper bin window. With the implementation of appropriate preventative measures, the automated assignment of allele designations by Genotyper® has proven to be very reliable. The reliability of this feature will prove a strong asset in databasing operations.