USE OF LOCUS SPECIFIC BRACKETS IN CALIBRATION OF Y CHROMOSOME STR ALLELES

Debang Liu, Xiang Wen Mang. Leilani Witt and Peter Dau
Oligotrail, LLC, Evanston. IL

Multiplex PCR amplification of Y chromosome STR alleles offers forensic science the opportunity to independently analyze the male DNA component from crime scene mixtures. Individual Y-STRs are relatively uninformative compared to autosomal STRs, because of the presence of only one allele at each STR locus and their lack of recombination with homologous chromosome from the female parent. Therefore, the measurement of many multiplexed Y-STR loci is desirable in order to increase their power of discrimination. Because the overall mutation rate of Y-STRs is comparable to that of autosomes, some are sufficiently polymorphic to make them useful in forensic science.

Locus specific brackets (LSB) make excellent calibration markers because they are derived directly from a target locus by means of recombinant DNA technology and designed to contain just 1-3 fewer or more repeat units than all known common alleles of that locus. Therefore, when LSB are loaded into a sample lane together with the PCR amplicons from their target loci, they will migrate in register just before and after the target alleles of their unique locus of origin to provide excellent calibration of their fragment lengths during electrophoresis. The calibration accuracy is stabilized against run-to-run variation by a standard electrophoresis of LSB together with 2 alleles from each locus as part of each group of sample runs. Importantly, since LSB are labeled with the fluoropore of their target locus the fluorophore employed in other calibration systems to label the internal lane standard is made available to label target alleles, which allows a shortening of STR fragment lengths.

In the present work LSB were amplified from *extended templates* of 10 Y-STR loci created by joining the central repeat regions to 200-300 bp of both 5' and 3' unique flanking sequence. Extended templates provided sufficient binding sites for different pairs of primers to amplify the appropriate LSB for a variety of PCR multiplexes designed during the research.

The multiplexed STR loci comprised the European haplotype, which are the 8 best studied Y-STRs (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, and DYS385), and the highly polymorphic YCAII locus, which together with the other 8 loci, form the European extended haplotype. To this group we added another informative and frequently employed locus, DYS388. Together these 10 loci yield 12 polymorphic STR products in a multiplex PCR reaction, because the YCAII and DYS385 primer pairs each amplify two independently polymorphic STR products from reduplicated sites on the Y chromosome. After electrophoresis alleles are called from their migration times and LSB calibration by software program developed for this purpose.