Experiences with the AmpF/STR® Yfiler® PCR Amplification Kit on an Asian Population
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The singular male Y-chromosome forms an important part of the genetic landscape of human variations with its unique biology, mutation and mechanism of inheritance. Microsatellites or short tandem repeats on the Y-chromosome (Y-STRs) have utility in forensic human identification beyond the conventional autosomal STRs. The strongest advantage of using Y-STRs is its ability to type even from a very limited amount of compromised male DNA in an overwhelming background of female DNA, thus avoiding the physical loss during direct extraction and more importantly, the masking of weak male alleles that is commonly and frustratingly observed using a standard STR system. Unlike autosomal STRs, the haploid Y-STRs can also simplify to a certain degree complex mixtures which are comprised of a number of male contributors in rape and sexual assault crimes. For such mixtures, using a Y-STR system can indicate the minimum number of male perpetrators involved.

Recently, Hansen and Ballantyne (2006) reported that there are over 417 Y-STR loci comprehensively annotated on the Y-chromosome (1), but only a small number of Y-STR genotyping systems are currently available to the forensic community. The Forensic DNA/Serology Laboratory of the Department of Chemistry in Malaysia validated and successfully implemented the AmpF/STR® Yfiler® PCR Amplification Kit for forensic casework in November 2005. This megaplex co-amplifies nine European-minimal loci (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS385a/b), two SWGDAM-extended loci (DYS438, DYS439) and six new loci (DYS456, DYS458, DYS635 or Y-GATA C4, Y-GATA H4, DYS437 and DYS448) in a single reaction. To date, the number of rape and murder cases analyzed in our laboratory using Yfiler® after standard STR analysis with the AmpF/STR® Identifiler® PCR Amplification Kit analysis has exceeded 100 cases. Rape and sexual assaults form the major bulk of the casework received by the laboratory, amounting to 45% of the total caseload in 2005. From our experience, Y-STR haplotyping has been most successful in retrieving male profile(s) from intimate samples such as vaginal, rectal and breast swabs/smears, bite marks on the body, tape-lifts of the pubis area and nail clippings of the female victim. For such samples, encountering a mixture of male and female DNA types is expected. While the STR profiles are often complicated due to overlapping contributions from the excessive female DNA present, Y-STR profiles can offer a simpler interpretation by eliminating the victim’s contribution.

At least 105 casework Y-STR profiles have been reviewed and this includes a variety of cases such as paternity, missing person, rape, murder, assault, robbery, etc. Prior to implementation in casework, a population study was carried out on 980 male individuals from the three major ethnic groups in Malaysia, comprised of 334 Malays, 331 Chinese and 315 Indians. The population data showed that the duplicated locus DYS385 has the greatest diversity values across all three groups (haplotype diversity, DH>0.950), while the highest single locus diversity for each of the groups was different. DYS448 was the most variable for the Malays (DL=0.808), DYS635 for the Indians (DL=0.829), and DYS458 for the Chinese (DL=0.815). Interestingly, these three most polymorphic Y-loci (DYS448, DYS635 and DYS458) were among the six new Y-STR loci that have been incorporated into the Yfiler® kit. This means that in addition to the minimal haplotype’s 9-loci and the SWGDAM 2-loci, the inclusion of DYS448, DYS635 and DYS458 renders the Yfiler® multiplex highly discriminating for forensic applications amongst the Asian groups.

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Both the database haplotypes and casework haplotypes showed allelic variants at several Yfiler® kit loci. Incidentally, the three most polymorphic loci, i.e. DYS448, DYS635 and DYS458, also showed the greatest number of microvariants. Other loci showing microvariants were DYS456, DYS391, DYS392, DYS437, DYS438 and Y-GATA H4. Allele 19 at DYS635 is the most frequently encountered variant allele so far as it has been observed in 37 of the database haplotypes and 7 of the casework haplotypes. Microvariants are important as they can increase the evidential value of the DNA profile in that it makes the profile more uniquely discriminating, but because it is not genotyped by the allelic ladder, sequencing is required to investigate the variation within the repeat and flanking regions.

The highly polymorphic DYS385 is not only a duplicated locus (one or two peaks), it can also be a triplicated locus (three or four peaks) resulting in several allelic peaks at this locus. This phenomenon can result in a limited use of DYS385 in resolving mixed profiles having two or more male sources. This is because two or more peaks for a single male source may then be observed. So far we have detected 4 males in the database haplotypes exhibiting DYS385 triplication (3 showed three peaks and 1 showed four peaks) and 4 males from the casework haplotypes with DYS385 triplication (all 4 showed three peaks). Interestingly six of the eight males with DYS385 triplications are from the Chinese population group. Duplication was also observed at another Y-locus (DYS439) in the casework haplotypes, but DYS385 is by far the locus that most frequently shows duplications.

Another type of non-standard alleles observed with the Yfiler® kit are null alleles (dropout of Y-STR alleles). These are observed at DYS458, DYS392, DYS389I, DYS389II, DYS439, DYS448 and Y-GATA H4. The locus with the largest number of nulls is DYS458, as seen in 20 males from the Malaysian population to date (12 males from the database and 8 males from casework). Out of these 20 nulls, 14 were from the Indian population group and 6 from the Malay population group. This null is associated with an Amelogenin Y-deletion recently reported by Jobling et al. (2007) to be caused by a 2.5-4 Mb TSPY (testis-specific protein Y-linked) mediated deletion on the short-arm of the Y-chromosome (3). Consequently the Amelogenin Y-allele is absent in these individuals and results in false genotyping of these males. Figure 1(A) below shows the STR profile of one of the deleted males which was amplified with the Yfiler® kit to give a Y-STR haplotype shown in Figure 1(B).

It is interesting to note that while DYS385 chromosomal locus duplication is mostly observed in the Chinese group, Amelogenin Y-deletions are only significantly observed in the other two groups, the Indians and the Malays.

Despite the presence of variant and non-standard alleles, the Yfiler® system has demonstrated forensic usefulness in the Malaysian population, where resolution of DNA mixtures and confirmation of Amelogenin nulls merits the application of Y-STR analysis.
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References:

