B162 Characterization of a Novel Stutter Product in the Y-STR Marker DYS392 a nd a Rare Polymorphic Variant in the DYS456 Homologue Identified Using the AmpF/STR Yfiler™ PCR Amplification Kit

Lori K. Hennessy, PhD, and Chien-Wei Chang, PhD, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, CA 94404; Bruce Budowle, PhD, FBI Laboratory, 2501 Investigation Parkway, Quantico, VA 22135; and Lisa Calandro, MPH, and Julio Mulero, PhD, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, CA 94404*

The goal of this presentation is to share the results obtained on the characterization of the novel stutter product in the Y-STR marker DYS392 and a rare SNP variant in the DYS456 homologue identified AmpFlSTR Yfiler™ PCR Amplification Kit.

This presentation will impact the forensic community and/or humanity by providing additional information to guide forensic scientists in the interpretation of data when using the AmpFlSTR Yfiler™ PCR Amplification Kit for Y chromosome STR analysis.

Y-chromosome short tandem repeat (STR) markers yield a high degree of confidence that only the male contributor is being analyzed in male-female mixtures. The AmpFlSTR Yfiler™ PCR Amplification Kit is a commercial multiplex system designed for the simultaneous amplifi- cation of 17 Y-STR markers (DYS19, DYS385a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS635 (formerly known as Y GATA C4) and Y GATA H4). A by-product of the amplification of the trinucleotide repeat locus DYS392 is the formation of N-3 and N+3 stutter products. Sequence analysis of the novel N+3 stutter band demonstrates that its sequence is one TAT repeat longer than that of the corresponding main allele. Both N-3 and N+3 stutter percentages increased as (1) the main allele repeat number increased, as (2) the magnesium concentration was increased in the reaction or if (3) the initial amount of DNA template was decreased. Since both stutter products behave in a similar and reproducible fashion, it is proposed that the same rules that apply to the interpretation of N-3 stutter products could be applied to N+3 stutters.

During an extensive multi-population study with Y-STR loci amplified using the AmpF/STR Yfiler™ PCR amplification kit, amplifi- cation of a 71-bp fragment was observed in 2.32% of the male samples ana- lyzed (N=3141). By direct sequencing of this fragment, it was determined that the primer binding sequences were identical to that of the DYS456 locus. A T to G single nucleotide polymorphism (SNP) enabled amplifi- cation of the 71-bp fragment. The SNP is located within an X-Y homol- ogous region at Xq21.31 and was observed with the highest frequency within the African American and Sub-Saharan African populations in this study. Presence of the SNP on the X chromosome did not interfere with the reliability of typing the DYS456 locus and the other Y STR loci typeable using the AmpFlSTR® Yfiler™ PCR amplification kit. Full profiles in a mixture of male: female at 1:4000 were obtained using the current config- uration of the AmpF/STR® kit even in the presence of female DNA con- taining the G variant.

In this study, the novel stutter product at the DYS392 locus and a new, rare SNP variant in the DYS456 homologue have been characterized. These results provide additional information to guide forensic scientists in the interpretation of data when using the AmpF/STR® Yfilerä PCR Amplification Kit for Y chromosome STR analysis.

Y-STR, Genotyping, SNP

 ** Presenting Author*