CHARACTERIZATION OF N+4 STUTTER IN PROFILER PLUS AND COFILER AMPLIFICATION

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PCR amplification is widely used in the field of forensic science. In the course of PCR amplification of STR sequences an artifact is created, known as stutter, which is thought to occur by sequence mis-alignment (Gibb, 2009). N-4 stutter is thought to occur when the parent strand loops out from the daughter strand during synthesis. As a result, PCR products one repeat less than the parent strand are produced. The same model may also be used to explain N+4 stutter in which products one repeat unit longer than the parent strand are produced (ibid). N+4 stutter has been characterized in the SGM Plus PCR kit previously, but there are few reports characterizing N+4 stutter resulting from the Profiler Plus and Cofiler amplification kits from Applied Biosystems. Understanding N+4 stutter in amplification systems is important for the analysis of mixtures.

We analyzed a combined 2,196 Profiler Plus and Cofiler amplicons generated from reference samples at a threshold of 30 relative fluorescence units (RFU) to determine the incidence of N+4 stutter. We found 142 amplicons (~6%) that displayed N+4 stutter. Interestingly, some batches of samples exhibited a higher incidence of n+4 stutter in comparison to the average (average 4, median 2, standard deviation 7 samples/batch). Two of the 44 analyzed batches had a rate of n+4 stutter more than three standard deviations above the average. To better understand factors contributing to N+4 stutter, we tested different thermal cyclers and capillary electrophoresis (CE) instruments used during sample processing, various lots of reagents, the age of the polymer, and the number of injections on the capillary. We found no correlation between these factors and the rates of N+4 stutter. We also examined the composition of the 13 CODIS core loci to determine whether pure versus interrupted repeats, G/C content, or the presence of the repeat unit within an *Alu* element could be a contributing factor to N+4 stutter. We found no association between the loci with high rates of positive stutter and their G/C content, repeat composition, or location within an *Alu* element.

Of the loci that are shared between the SGM Plus amplification kit and the Profiler Plus/Cofiler kits, the rates of N+4 stutter observed differed between kits. The loci producing the highest rates of N+4 stutter with the SGM Plus kit were D21S11 (33%), D18S51 (22%), vWA (9%), D3S1358 (8%), D16S539 and FGA (5% each). The other SGM Plus loci produced two percent or less of the total number of stutter occurrences. The most common loci to produce N+4 stutter with the Profiler Plus/Cofiler kits were D3S1358 (39%), D16S539 (16%), D21S11 and D5S818 (8% each), FGA and CSF1PO (6% each). The other loci produced five percent or less of the total stutter occurrences with the exception of TH01 and TPOX where no instances were observed. Of the 67 instances of forward stutter in D3S1358, 52 were from Cofiler amplifications while only 13 were from Profiler Plus amplifications, even though the same known samples were used for both amplifications. The observation that the same loci in different amplification kits produce different rates of stutter suggests that amplification conditions may be a factor in the formation of N+4 stutter products.

This presentation will further delineate the average, range, and standard deviation of the heights of the stutter peaks and the overall occurrence of forward stutter. These rates will also be compared to those reported in the study by Gibb et al.

REFERENCES

Gibb, A.J., *et. al,* (2009) Characterisation of forward stutter in the AmpFlSTR SGM Plus PCR, *Science and Justice*,49: 24-31.