An Evaluation and Comparison of Locus-Specific and Allele-Specific Alternate Stutter Artifacts Frequently Encountered in GlobalFiler™ DNA Profiles

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INTRODUCTION

Forensic DNA analysts commonly encounter stutter during interpretation. Stutter, a DNA artifact caused by strand slippage of Taq polymerase during polymerase chain reaction (PCR), most commonly results as a deletion of a single repeat unit. Stutter has the potential to create serious challenges during DNA interpretation especially when the evidence profile is a mixture of multiple individuals. Misinterpreting a stutter artifact can lead to the false inclusion or exclusion of a person of interest. Therefore, it is critical that stutter is thoroughly understood and accurately identified when present. Both locus-specific and allele-specific stutter have been studied and stutter percentages have been developed to help predict and identify stutter when the stutter peak is one repeat unit shorter in length than parent allele. However, limited information exists to assist with the accurate identification of alternate stutter peaks that are not one repeat unit less than the parent allele.

METHODS

- Analyzed 165 single source GlobalFiler™ DNA profiles provided by ThermoFisher Scientific with stutter filters turned on and off using GeneMapper® IDX v 1.4
  - 1 ng total of DNA
  - Encompassed African American, Caucasian, and Hispanic Populations
- Identified possible stutter peaks at each locus excluding additive stutter or stutter peaks that may overlap with other artifacts
- Documented the following information for each occurrence: parent alleles, parent allele RFU's, stutter artifacts, stutter artifact RFU's, and stutter type (*i.e.,* N – 2, N – 4, etc.)
- Calculated allele-specific stutter percentage
  - Stutter % = (RFU of Stutter Peak/ RFU of Parent Allele) x 100
- Plotted the most prevalent stutter locations found and further analyzed using Microsoft Excel®
- Based on trends observed, determined whether an allele-specific stutter filter or a locus-specific stutter filter was more appropriate at the stutter locations evaluated

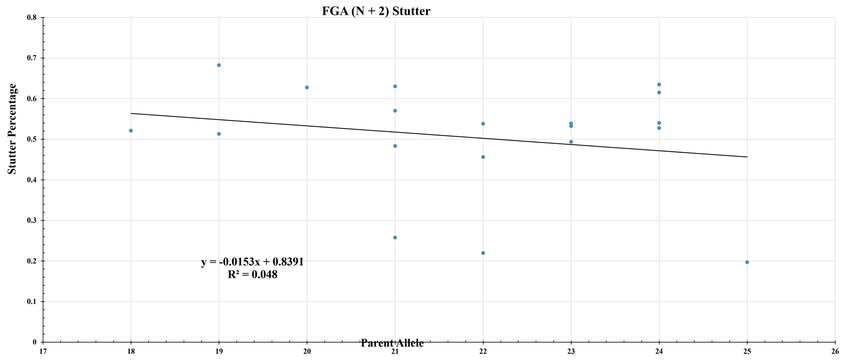
RESULTS

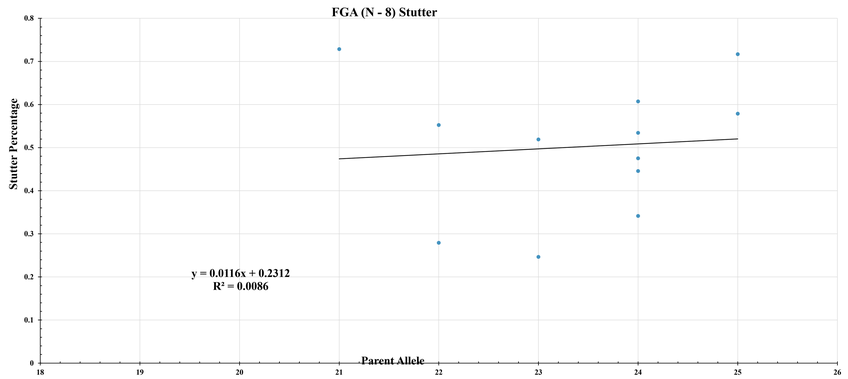
Table 1: Frequency of most prevalent alternate stutter artifacts at FGA, SE33, and D1S1656 across 165 single-source DNA profiles from the African American, Caucasian, and Hispanic populations.

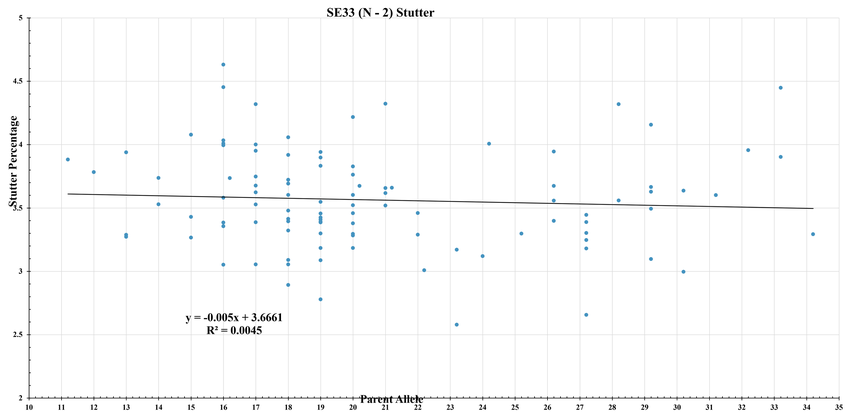
Locus	Alternate Stutter	Number of Observations		
		African American (n = 55)	Caucasian (n = 55)	Hispanic (n = 55)
FGA	N + 2	19	46	28
FGA	N – 8	13	25	19
SE33	N – 2	102	101	93
SE33	N – 6	78	48	63
SE33	N – 8	78	55	67
D1S1656	N – 2	78	80	91

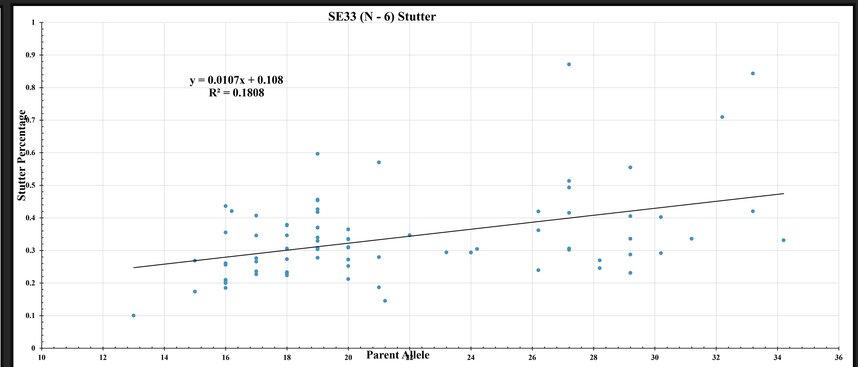
RESULTS

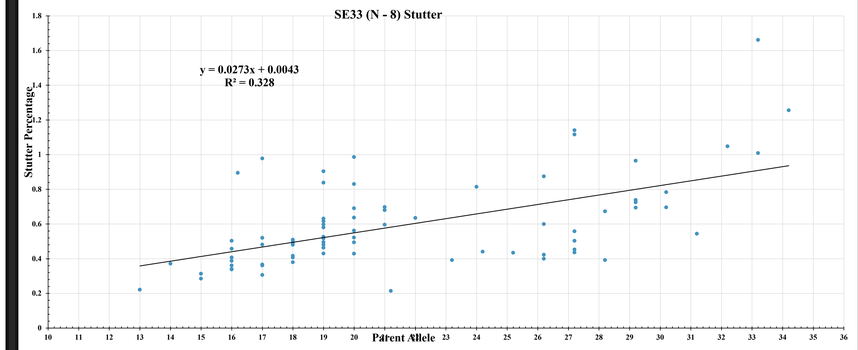
Figures 1 – 6 contain separate scatter plots for each locus and alternate stutter artifact for the African American samples with Parent allele (x-axis) vs. Stutter Percentage (y-axis). The data displayed similar characteristics across all three ethnic population groups.

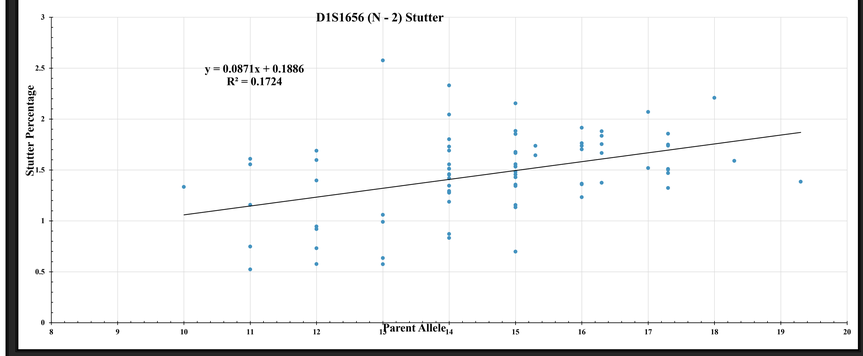












DISCUSSION AND CONCLUSION

The line of best fit slope indicated that allele-specific stutter filters for FGA (N – 8), SE33 (N – 6), SE33 (N – 8), and D1S1656 (N – 2) were more appropriate to accurately identify stutter. Conversely, for FGA (N + 2) and SE33 (N – 2), the line of best fit slope indicated that locus-specific stutter filters may be used. Overall, the results demonstrate the importance of implementing allele-specific stutter filters for alternate stutter at some loci rather than locus-specific stutter filters. Allele-specific stutter filters will allow for the more accurate identification of alternate stutter products and decrease the chances of incorrectly labeling a stutter peak as a true allele or incorrectly removing a true allele as stutter.

ACKNOWLEDGEMENTS

The authors would like to thank ThermoFisher Scientific for providing the raw data used to conduct this project and SoftGenetics® for advising on this project.