Analysis of (N-4)bp STR Repeat Slippage on Low-quantity DNA Samples with PCR Boost

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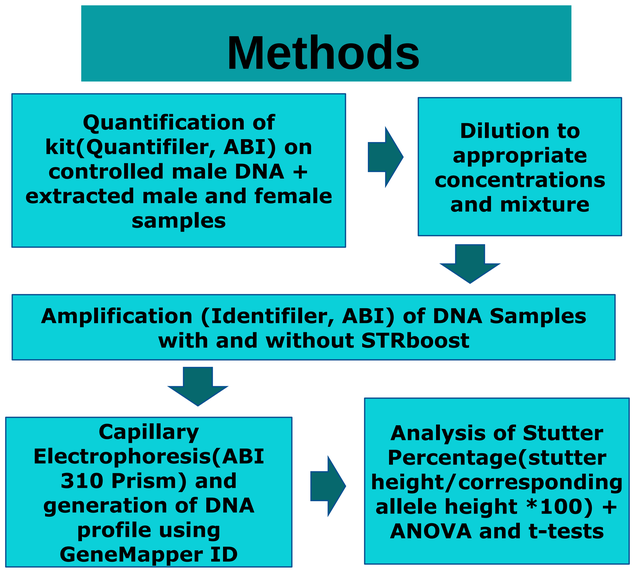
Abstract

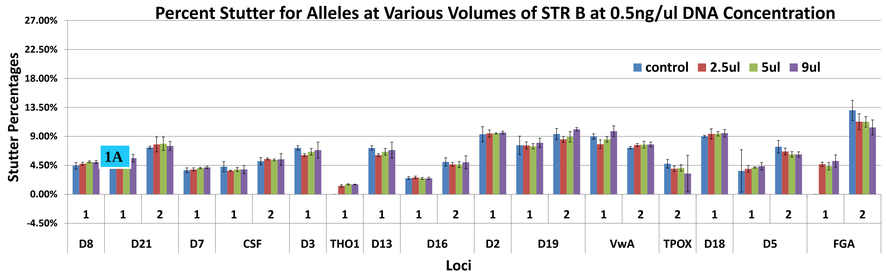
Forensic DNA profiles are based on small repetitive nucleotide sequences called short tandem repeats_(STR) that vary by size in the population. Due to stochastic effects, low copy number (LCN) DNA samples produce more random fluctuations and artifacts than larger quantities of DNA. One artifact, called repeat slippage or stutter, results due to strand slippage during the PCR process. Stutter can complicate interpretation of profiles, especially in mixtures. Thus, minimizing amplification of stutter products is important when analyzing LCN samples.

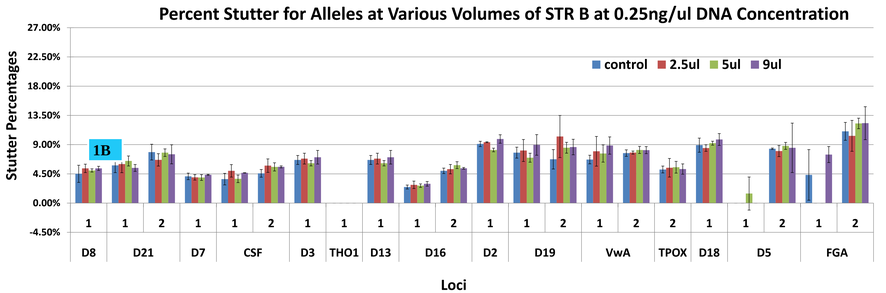
A new PCR enhancer, STRboost_(SB), has been used to enhance amplification from low-quantity samples. **The purpose of this study is to evaluate the percentage of stutter formation with various volumes of SB. The hypothesis is that amplification of low-quantity DNA samples with SB will result in higher peak heights with no significant change in stutter percentage**. Human male DNA was diluted to 0.5ng/µl, 0.25ng/µl, and 0.125ng/µl. Extracted male and female DNA samples were quantified using qPCR and diluted to produce a 0.5ng/µl mixture of 9:1 female to male ratio. The DNA samples were amplified in triplicates using the AmpFISTR Identifiler STR Multiplexing kit_(ABI) using SB at three volumes: 2.5µl, 5.0µl, and 9.0µl. The mixture sample was tested at only 9ul of SB. Amplicons were separated by capillary electrophoresis and a genetic profile was generated using GeneMapper ID. The stutter percentages in the triplicate runs at each concentration were compared using the Single-Factor ANOVA and Independent Two-Sample t-test with alpha being 0.05.

Methods



Results





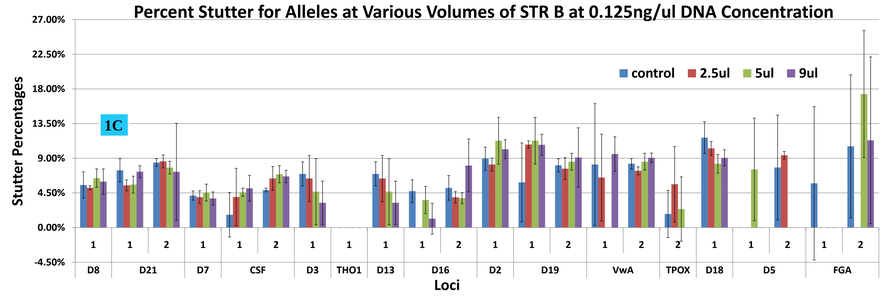


Figure 1a-c: Average allelic stutter percentages at 0.5ng/ul, 0.25ng/ul, and 0.125ng/ul at different loci and at four conditions of STRboost. Some alleles show two peaks(heterozygous) and some show one peak(homozygous). Not all alleles showed stutter peaks.

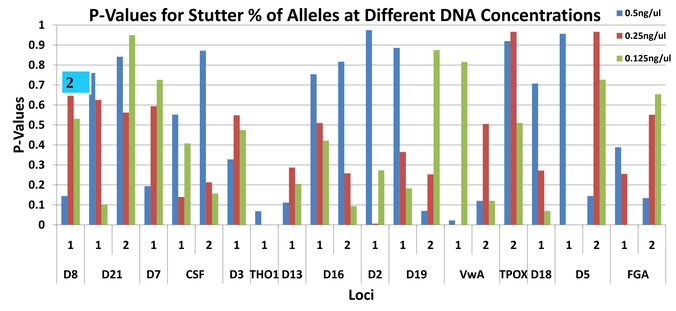


Figure 2: ANOVA and t-test p-values determined from comparison of triplicate runs at various volumes of SB. The t-test was used for some loci that showed reproducible stutter peaks at only two volumes of STRboost.

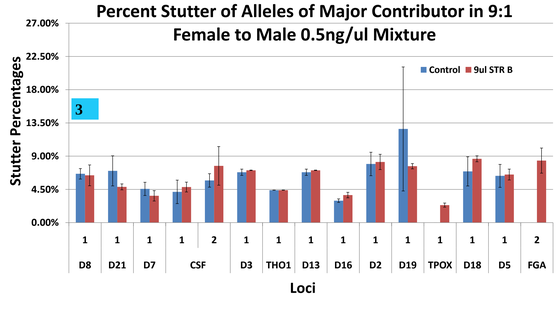
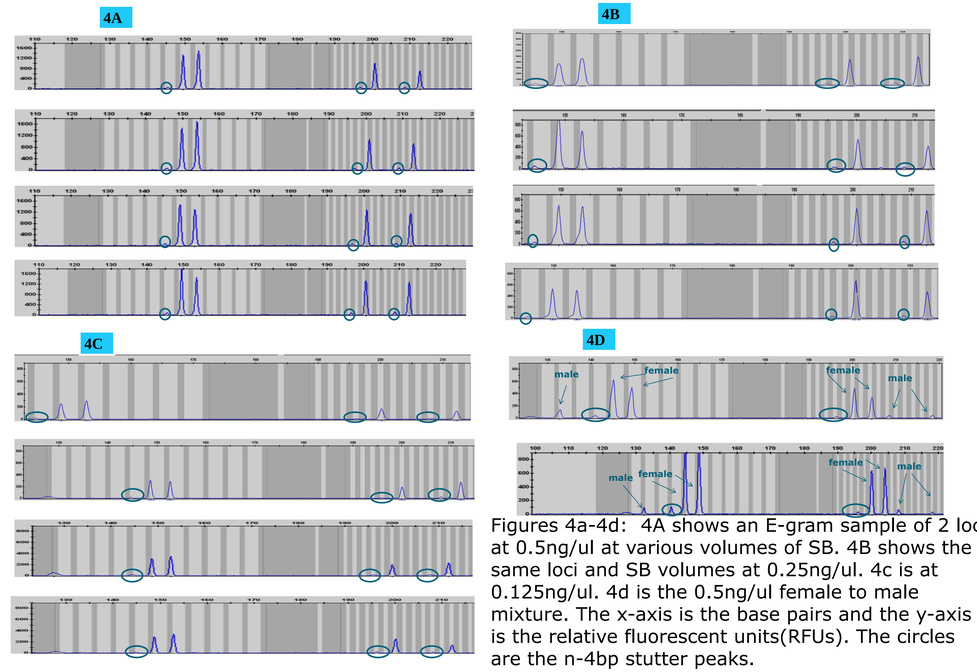


Figure 3: Average allelic stutter percentages at of major contributor(female) in 0.5ng/ul mixed sample of 9:1 female to male at 0ul and 9ul of STRboost.



Figures 4a-4d: 4A shows an E-gram sample of 2 loci at 0.5ng/ul at various volumes of SB. 4B shows the same loci and SB volumes at 0.25ng/ul. 4c is at 0.125ng/ul. 4d is the 0.5ng/ul female to male mixture. The x-axis is the base pairs and the y-axis is the relative fluorescent units(RFUs). The circles are the n-4bp stutter peaks.

Conclusions

- The proportional enhancement of stutter peaks and the corresponding true allele supports the hypothesis.
- Single-source DNA samples and the 0.5ng/µl mixture show that the allele and stutter heights increased with no significant change(p-values> 0.05) in stutter % in most of the loci.
- Stochastic effects greatly increased at lower concentrations of DNA causing the p-values to approach alpha.
- Highest peak heights (sensitivity) were obtained between 5-9µl of the enhancer.
- Preliminary results of the 0.5ng/µl mixture show higher stutter percentages than in the 0.5ng/µl single-source sample.
- Explanation for enhancement may due to the fact that stutter sequences only differ from the true alleles by one repeat (4bp).

Acknowledgements

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